HISTOLOGY AND HISTOPATHOLOGY

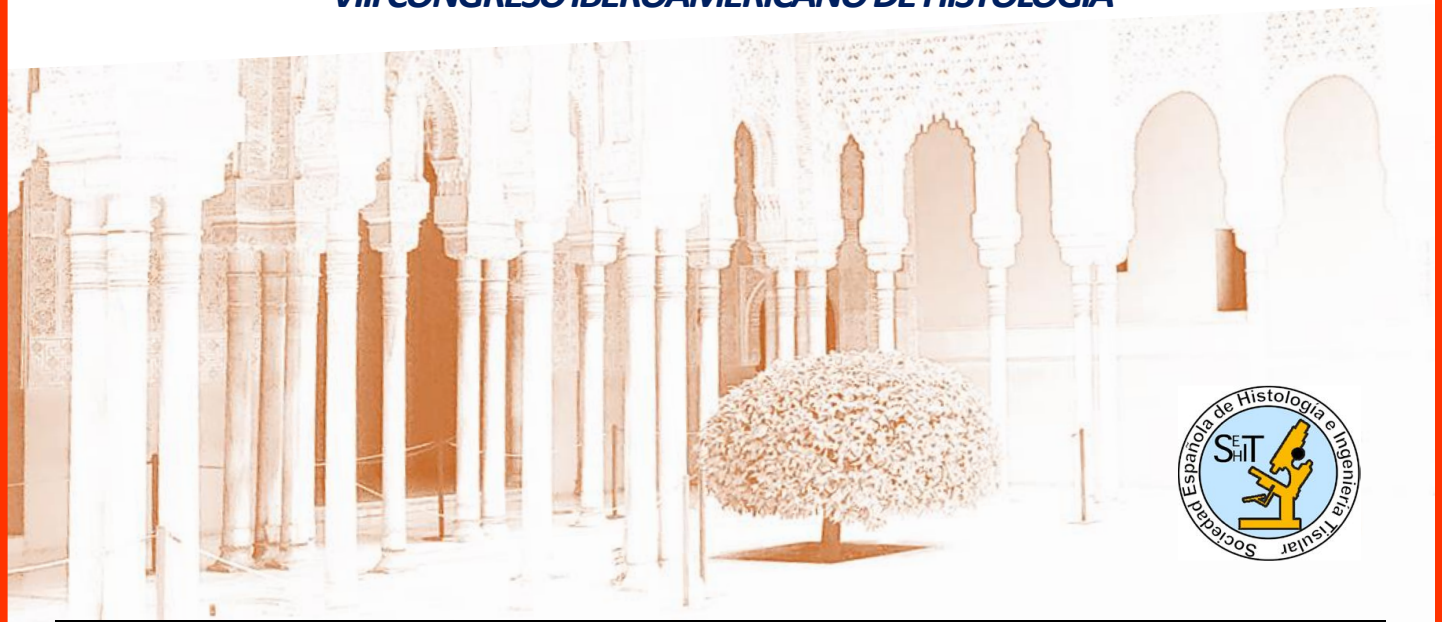
Cellular and Molecular Biology

Volume 37 (Supplement 1), 2022

XXI CONGRESO DE LA SOCIEDAD ESPAÑOLA DE HISTOLOGÍA E INGENIERÍA TISULAR

IX INTERNATIONAL CONGRESS OF HISTOLOGY AND TISSUE ENGINEERING

VIII CONGRESO IBEROAMERICANO DE HISTOLOGÍA

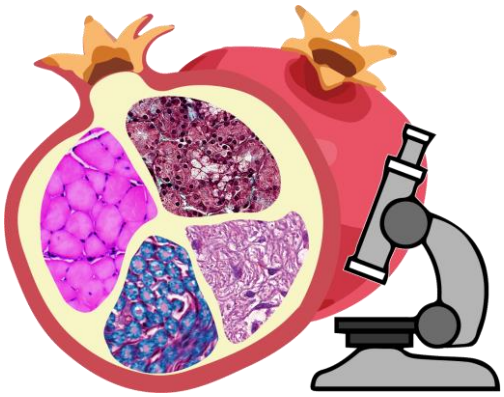


Online ISSN 1699-5848
Print ISSN 0213-3911

XXI CONGRESO DE LA SOCIEDAD ESPAÑOLA DE HISTOLOGÍA E INGENIERÍA TISULAR

IX INTERNATIONAL CONGRESS OF HISTOLOGY AND TISSUE ENGINEERING

VIII CONGRESO IBEROAMERICANO DE HISTOLOGÍA



Histology and Histopathology
From Cell Biology to Tissue Engineering

Submission Guidelines

1. Manuscript types

Original articles. There is no page limit, but 15-25 printed pages are the most usual. There is no figure limit, but they should be strictly necessaries. There is no limit for tables, but they should be strictly necessaries and easy to understand and reproduce in the published paper. Supplementary material should be restricted to extensive figures and tables not necessaries for the interpretation of the results, but that could give more information to readers interested in some particular procedure or method.

Reviews. Same comments that for original articles. They should be written by experienced authors with several published articles in the subject revised.

Invited reviews. Same comments than for original articles. It can be submitted after invitation of the editor of the Journal. They are free or charges.

2. Manuscript submission

Please make your submission online at https://www.editorialmanager.com/hh/default.aspx. The text document must be saved as Word or RTF format. Tables must be included in the text document. Figures must be saved in the formats and at the resolution below indicated.

2.1. Submission of a manuscript implies:

1) The work described has not been previously published, except in abstract form in a Congress

2) The work described is not under consideration for publication anywhere else

3) The publication of manuscript in Histology and Histopathology is approved by all co-authors

4) The publisher will not be held legally responsible should there be any claims for compensation

5) The authors should have obtained permission from the copyright owner for any figures or tables previously published. They should retain the permission documents and the previous publication should be properly cited. Any material received without such evidence will be assumed to originate from the authors.

6) Authors must maintain all the raw data. They could be asked by the Journal if necessary. If they are not suitable, the article could be retracted.

2.2. Manuscripts should have:

2.2.1) A concise **cover letter**.

2.2.2) A **title page**. Title page should contain the title, author list (given and family name, but not degree), affiliation, contact details for corresponding author, key words (different to those in the title) and short title.

2.2.3) An **abstract**. About 250 words.

2.2.4) Main text for original articles

Do not divide words at the end of lines. Pages should be numbered.

References to the literature should be cited in the text by the name of the author(s) followed by the year of publication. In cases in which there are more than two authors, only the first is named, followed by "et al.". Examples: Smith (1980) reported that...; (Smith, 1980, 1982); (Smith and Tanaka, 1980); (Smith et al., 1980). Suffixes a, b, etc., should be used following the year to distinguish two or more papers by the same author(s) published in the same year; example (Smith, 1981a). When two or more references are included in the same bracket, they must be quoted in the chronological order; example (Smith, 1980; Bell et al., 1984).

Main text for original articles should contain the sections:

a) List of abbreviations (if any)

b) Introduction

c) Material and methods

d) Results

e) Discussion

f) Acknowledgements, including funding sources

g) A conflict of interest statement

h) The reference list should be in alphabetical order.

References to articles in periodical publications must include: Names and initials of all authors, year of publication, complete title of paper, name of journal (abbreviated in accordance with PubMed), number of volume, and first and last page numbers. Example: Morita T., Suzuki Y. and Churg J. (1973). Structure and development of the glomerular crescent. Am. J. Pathol. 72, 349-368.

Reference to books must include: Name and initials of authors, year of publication, full title, edition, editor, publisher, place of publication and page numbers. Example: Powell D. and Skrabanek P. (1981). Substance P. In: Gut Hormones. 2nd ed. Bloom S.R. and Polak J.M. (eds). Churchill Livingstone. Edimburgh. pp 396-401.

i) Tables. Numbered in arabic

j) Figure legends. Including experimental group, technique, meaning of arrows or letters in the figures, magnification in the form of scale bars and any other information that help to understand the figure.

2.2.5) Main text for reviews. Like original articles, except for the sections Introduction, Material and methods, Results and discussion that are not mandatory. The authors are free to select different sections.

2.2.6) As separate files. **Illustrations** should not exceed 17.8 x 22.2 cm. The Editor reserves the right to reduce or enlarge the illustrations. Apply figure numbers to the lower left-hand corner of each photograph and the scale bar at the lower right-hand corner. Images should be TIFF file format, preferentially, although other formats could be useful (jpg, ppt, etc). Black and white figures must be at gray scale. Color figures should be preferentially in CMYK, but RGB is also allowed. Line art files must have a 500dpi resolution, while other images must have a 300dpi resolution.

3. Article processing charges (APCs)

The articles are published under a Creative Commons (CC BY) license. The price is 1,600.00 euros (tax excluded) independently of the number of color figures and pages.

4. Copyright and license term

The copyright remains with the author. author(s) agree to publish the article under the Creative Commons Attribution License CC BY

5. Review process

An initial assessment will be made by the Editor-in-Chief following submission of your manuscript. This process considers whether the submitted work falls within the scope of the Journal and is of initial interest and/or scientific worth to merit possible publication. Manuscripts that enter the review process may be assigned to the Editor-in-Chief, another Editor or a member of the Editorial Board who invites reviewers (normally two-three external reviews are sought). The reviewers' evaluations and Associate Editor's comments are submitted to the Editor-in-Chief (or the relevant Regional Editor) to inform a final decision. We aim to convey a decision within four weeks of the receipt of the manuscript. The review process is simple-blind type.

The Editor-in-Chief based on the reviewers' evaluations and with possibly with the help of a member of the Editorial Board will advise authors whether a manuscript is accepted, requires revision, or is rejected. Revisions are expected to be returned within a fixed time, depending on the extension of the modification suggested by the reviewers. Manuscripts not revised within this time are subject to withdrawal from consideration for publication unless there are extenuating circumstances. Please note that some manuscripts will have to be rejected on the grounds of priority, interest, journal balance and available space. Invitation to submit a revised manuscript does not imply that acceptance will automatically follow. The decision of the Editor-in-Chief is final. If, however, authors dispute a decision and can document good reasons why a manuscript should be reconsidered, a rebuttal process exists. In the first place, authors should write to the Editor-in-Chief outlining their case.

6. Post-acceptance procedures

Accepted Articles. Accepted Preprints version (authors' manuscripts of accepted articles, prior to copyediting, page layout and proofing) are available shortly after the day of acceptance for publication in our web site and in some indexing sites.

Proofs. Proofs in PDF format will be sent to the corresponding author for checking. This stage is to be used only to correct errors that may have been introduced during the production process. Prompt return of the corrected proofs, preferably within three days of receipt, will minimize the risk of the paper being held over to a later issue.

Histology and Histopathology
From Cell Biology to Tissue Engineering

Committees

Comité Organizador

Presidente
Crespo Ferrer, Pascual Vicente

Vicepresidente
García López, José Manuel

Vocales
Sánchez Quevedo, María del Carmen
Alaminos Mingorance, Miguel
Cañizares García, Francisco Javier
Fernández Segura, Eduardo
Arrebola Vargas, Francisco
Garzón Bello, Ingrid Johanna
Carriel Araya, Víctor Sebastián
Martín Piedra, Miguel Ángel
Campos Sánchez, Fernando
Chato Astrain, Jesús
García García, Óscar Darío
Rodríguez, Ismael Ángel
Oyonarte Gómez, Salvador
Sola García, Miguel
Sánchez Porras, David
Blanco Elices, Cristina
Ortiz Arrabal, Olimpia
Galiano Simón, Hermelinda
Bermejo Casares, Fabiola
De la Cueva Batanero, Paloma

Comité Científico

Presidente
Campos Muñoz, Antonio

Vocales
Alfonso Rodríguez, Camilo *(Universidad Antonio Nariño, Colombia)*
Alonso Varona, Ana *(Universidad del País Vasco, España)*
Álvarez Vázquez, Pilar *(Universidad Complutense, España)*
Arriazu Navarro, Riánsares *(Universidad CEU-San Pablo, España)*
Buján Varela, Julia *(Universidad de Alcalá, España)*
Carriel Araya, Víctor Sebastián *(Universidad de Granada)*
De Juan Herrero, Joaquín *(Universidad de Alicante, España)*
De Miguel Rodríguez, Manuel *(Universidad de Sevilla)*
D'Ottavio, Alberto Enrique *(Universidad Nacional de Rosario, Argentina)*
Durand Herrera, Daniel *(Univ. Michoacana de S. Nicolás de Hidalgo, Morelia, México)*
Fenoll Brunet, María Rosa *(Universidad Rovira i Virgili)*
Fernández Santos, José María *(Universidad de Sevilla)*
Fontanilla Duque, Martha Raquel *(Universidad Nacional de Colombia)*
García Caballero, Tomás *(Universidad de Santiago de Compostela, España)*
García Garza, Rubén *(Universidad Autónoma de Coahuila, México)*
García Gómez de las Heras, Soledad *(Universidad Rey Juan Carlos)*
García Honduvilla, Natalio *(Universidad de Alcalá, España)*
García Lorenzana, Mario *(Universidad Autónoma de Metropolitana, México)*
Garrosa García, Manuel *(Universidad de Valladolid, España)*
Godoy Guzmán, Carlos *(Universidad de Santiago, Chile)*
Gómez Torres, María José *(Universidad de Alicante, España)*
Guzmán García, Xochitl *(Universidad Autónoma Metropolitana, México)*
Hilario Rodríguez, Enrique *(Universidad del País Vasco, España)*
Jaimes Parra, Boris *(Universidad de Bucaramanga, Colombia)*
Jimena Medina, Ignacio *(Universidad de Córdoba)*
Junquera Escribano, Mª Concepción *(Universidad de Zaragoza)*
Madrid Cuevas, Juan Francisco *(Universidad de Murcia)*
Martín Lacave, Inés *(Universidad de Sevilla, España)*
Martínez Ramírez, Alfredo *(CIBIR, España)*
Mata Roig, Manuel *(Universidad de Valencia)*
Montuenga Badía, Luis *(Universidad de Navarra, España)*
Navarro Incio, Ana María *(Universidad de Oviedo, España)*
Pastor García, Luis *(Universidad de Murcia)*
Peinado Herreros, María de los Ángeles *(Universidad de Jaén, España)*
Peña Amaro, José *(Universidad de Córdoba, España)*
Redondo García, Eloy *(Universidad de Extremadura, España)*
Roma, Stella Maris *(Universidad Nacional de Rosario, Argentina)*
Ruiz Saurí, Amparo *(Universidad de Valencia, España)*
Sáez Crespo, Francisco José *(Universidad del País Vasco, España)*
Salido Pericaula, Mercedes *(Universidad de Cádiz)*
San Martín Henríquez, Sebastián *(Universidad de Valparaiso, Chile)*
Sancho-Tello Valls, María *(Universidad de Valencia)*
Santafé Martínez, Manel *(Universidad Rovira y Virgili, España)*
Santamaría Solís, Luis *(Universidad Autónoma de Madrid, España)*
Villaró Gumpert, Ana Cristina *(Universidad de Navarra)*
Ximenes Oliveira, Ana Celeste *(Fortaleza, Ceará, Brasil)*

Histology and Histopathology
From Cell Biology to Tissue Engineering

ROUNDTABLES

MESAS REDONDAS

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

THEMATIC ROUNDTABLES

Tissue Engineering and Advanced Therapies

Garzón I.[1,2,*], Alonso-Varona A.I.[3], García-Honduvilla N.[4], Meana A.[5] and Alaminos M.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] de Biología Celular e Histología, Facultad de Medicina y Enfermería, Universidad del País Vasco; [4] Departamento de Medicina y Especialidades Médicas, Facultad de Medicina, Universidad de Alcalá; [5] Fundación de Investigación Oftalmológica, Oviedo, Asturias; * Coordinador de la mesa redonda

It has been almost 30 years since the term “Tissue Engineering” was proposed as a novel discipline combining the use of cells, biomaterials and signaling molecules to generate bioartificial tissues and organs able to mimic native structures [1]. This interdisciplinary area has attracted widespread attention as an innovative and promising tool that can be used in basic scientific research and in regenerative medicine. In fact, several models of tissues and organs have been successfully transferred to the clinical setting and demonstrated to be potentially useful to replace lost or severely damaged tissues and organs.

Most tissues and organs generated by Tissue Engineering are considered by the European Medicines Agency as Advanced Therapy Medicinal Products (ATMP), and the regulatory frame associated to these tissues and organs is analogue to other types of medicines [2]. Once generated as ATMP, some of these products demonstrated to be capable of overcoming the drawbacks associated to organ transplantation, especially regarding the tissue availability and the possibility of generating these tissues using autologous cells that are not associated to the risk of immune rejection. This area is currently experiencing an exponential growth as alternative therapies for the regenerative treatment of different pathologies that do not have a currently available curative option.

This scientific session describes the current state of Tissue Engineering, from the fundamental concepts to its applications. Several applications are discussed in the field of bone, cartilage, cornea, skin and other human tissues, from the *ex vivo* generation of these tissues, to their clinical applications. The different biofabrication methods based on novel biocompatible biomaterials and alternative cell sources are discussed by experts in the field of Tissue Engineering. Moreover, the process of clinical translation of bioengineered tissues is complex and requires the exhaustive characterization of the novel tissues at the ex vivo and in vivo levels, along with the authorization of the national medicines agencies.

Based on the wide experience of the participants in this session, the key steps involved in the generation, characterization and clinical translation of bioengineered tissues are discussed, with an emphasis on the collaboration between basic researchers and clinicians.

Presentations:

Alonso-Varona A. “Ingeniería Tisular en la reparación de defectos osteocondrales”

García-Honduvilla N. “Utilidad de las células mesenquimales para la generación de tejidos mediante ingeniería tisular”

Meana A. "Retos pendientes en la producción de Epitelios Artificiales”

Alaminos M. “Medicamentos funcionalizados de Ingeniería Tisular”

[1] Langer R & Vacanti J. Science. 1993; 260: 920-926

[2] Cuende N, et al. Cytotherapy. 2014; 16(12):1597-1600

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

THEMATIC ROUNDTABLES

Teaching Histology

Sáez F.J.[1,*], Peña J.[2], Fernández D.[3], Bermúdez D.T.[4] and Carrascal E.[5]

[1] Dept. of Cell Biology and Histology, School od Medicine and Nursing, University of the Basque Country UPV/EHU, Leioa, Spain; [2] Dept. of Morphological and Social Health Sciences, School of Medicine and Nursing, University of Cordoba, Spain; [3] Dept. of Cell Biology, Histology and Farmacology, School of Medicine, University of Valladolid, Soria, Spain; [4] Dept. of Human Physiology, Human Histology, Pathological Anatomy and Physical and sports Education, School of Medicine, University of Malaga, Spain; [5] Dept. of Anatomy and Human Histology, University of Salamanca, Spain; * Round table coordinator.

In the 16th century there was a curious confrontation between the pilots of the ships in the West Indies Route and the cosmographers. The pilots, who were in charge of guiding the ships, used its own handcrafted and inaccurate nautical charts based on their personal experience to estimate the ship's course and position, while the cosmographers wanted them to use their mathematical methods and their precise nautical charts. In this dispute, the pilots asked to be left to use their own methods and not to innovate anything. This debate has a similar parallel in today's university teaching, where traditional teaching methods of proven efficiency coexist with innovative projects that aim to improve student learning.

For centuries, teaching in universities was centered on the master class. At a time when books were scarce, it was common for the professor to read a book so that the students could copy it. In fact, in some countries, professors are called lecturers. Teaching aids were scarce. Apart from books, the teacher could help himself by writing or drawing on the blackboard, and in some subjects, such as medicine, practical teaching could be done by studying the patients or by dissecting cadavers.

Nowadays, books are abundant and accessible. Technological progress has facilitated access to audiovisual media, and the rise of the Internet has globalized access to information, sometimes of dubious quality. In addition, new tools and social networks have emerged that are very popular among young people and allow rapid interaction in groups. On their side, the authorities are encouraging teaching innovation.

Is teaching innovation a solution? The speakers at this round table present their experiences. In Histology instruction, the observation of microscopic slides is essential. Is it possible to innovate in this field? Professor José Peña Amaro explains how to help students interpret a histological preparation. The difficulty that the beginner has in understanding microscopic images is mainly based on two reasons: the variability of the three-dimensional morphology of the cells and the extracellular matrix, and the use of different types of microscopy.

Recent technologies can be used to teach Histology. Podcasts are audio or video files available on demand. Professor Diego T. Bermudez Flores has developed a very low cost and standardized method for the development of podcasts that were produced during a semester with a high percentage of follow-up. His experience indicates that podcasting as a tool for teaching Histology is viable, sustainable, easy to implement and widely accepted by students.

Social networks can also be used to facilitate learning. Following this premise, Professor Diego Fernández Lázaro uses Pinterest as a tool to support his teaching work.

Finally, Professor Eliseo Carrascal, with 45 years of experience teaching Histology, in which he has combined traditional methods while being an innovator in the use of new technologies, tells us that Socrates, the father of philosophy, carried out an exclusively verbal teaching that created a school, and reflects on what was the secret of his method and on the current situation of teaching at the University.

To innovate or not to innovate in education, this is the question, with its supporters and its detractors. Who is right? As in many debates, all positions may be partly right. No method is infallible, nor does it guarantee learning success. Any teaching method can be successful if it takes into account the context and is applied appropriately.

Presentations:
Peña J. "Knowing how to see: how to look at a histological slide"
Fernández D. "Pinterest as a virtual tool for learning Histology"
Bermúdez D.T. "The podcast as an auxiliary tool for the teaching of Human Histology"
Carrascal E. "From Socrates to artificial intelligence"

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

THEMATIC ROUNDTABLES

Advances in Histotechnology

Martín-Lacave I.[1,*], López-Cepero J.M.[2], Carriel V.[3,4], Junquera C.[5] and García-Caballero T.[6]

[1] Dept. of Normal and Pathological Cytology and Histology; School of Medicine, University of Seville, Spain; [2] Dept. of Pathology, Cellular Biology and Histology. School of Medicine, University of Cadiz, Spain; [3] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [4] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [5] Dept. of Human Anatomy and Histology, School of Medicine, University of Zaragoza, Spain; [6] Dept. of Morphological Sciences, School of Medicine and Dentistry, Santiago de Compostela University, Spain; * Coordinador de la mesa redonda

In this symposium, the basic concepts of the use of different canonical and new staining methodologies for the diagnose and treatment of human diseases will be exposed.

The importance of classical silver impregnations methods will be analysed with a new scope. Most useful methods are based on ammoniacal silver solutions, whose exquisite sensitivity can be attributed to a nucleation step of metallic silver nanoclusters over some tissue component. Their selectivity can be adjusted by modifying fixation, or by adjusting first steps of the procedure and then to develop (enlarge) these nuclei to obtain nanoparticle growth by photographical methods (developers) or chemical reduction (formaldehyde). In spite of their sensitivity and selectivity, the chemical basis of initial clustering is not specific from a biochemical viewpoint (except autometallographic methods). Silver nanoparticles can be modified by photographic toning to change their number, size and composition (e.g.: Au for silver). Nanoparticles (10-100nm) can be characterized by their wavelength spectra. Río-Hortega's silver carbonate is the most versatile and useful method, and new advanced microscopy can be applied to revisit and validate the classical results, and to identify the substrate by colocalization with histochemical (or immune) methods, TEM, correlative, and analytical microscopy.

In tissue engineering (TE) is it important to confirm that the engineered tissues generated can resemble the structure, composition, and function of target native tissues. To characterize these bioartificial substitutes molecular, biomechanical, and histological methods are needed. Histology plays a key role as by using adequate methods it is possible to evaluate three main features: histological pattern, extracellular matrix/biomaterials structures and composition, and cell-related molecules and/or chemical components.

Histological methods are highly useful during the ex vivo characterization of these substitutes. However, histology is especially relevant in preclinical in vivo studies, allowing to confirm the therapeutic efficacy of the substitutes generated, the fate of the materials used and negative results.

The importance of the provided data by the transmission electron microscopy (TEM) in different topics of scientific research will be displayed. The ultrastructure of Interstitial Cells of Cajal (ICCs) will be analysed, cells in which a new organelle has been described for the first time: the primary cilium, a sensory structure with crucial functions in cell signalling. This fascinating nanostructure also appears at specific times in the life of immature neurons and glial cells.

The usefulness of TEM for studying the tumours originated from mutations in ICCs, the so-called GIST tumours (gastrointestinal stromal tumours), will be also reported. Concretely, the implication of primary cilia in the tumour process, as well as the formation of spherosomes. The presence of the primary cilium in various carcinomas questions the role of the primary cilium as a possible therapeutic target.

Finally, immunohistochemistry is a histological technique that revolutionized pathological diagnosis (the so-called "brown revolution"). Advances in immunohistochemistry such as automation/standardization, external quality controls, increased sensitivity, antibodies in the top ten in pathology, and recently appearing antibodies are reviewed.

Presentations:

López-Cepero J.M. "Back to the future: the silver impregnation methods"
Carriel V. "Usefulness of histological quality controls in tissue engineering"
Junquera C. "How Cells Talk to us through Transmission Electron Microscopy"
García-Caballero T. "Immunohistochemistry: What's up, doc?"

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

THEMATIC ROUNDTABLES

Current State of Histology

Garrosa M.[1], Noguera R.[2], Fernández Santos J.M.[3], Martín-Piedra M.A.[4] and Madrid J.F.[5]

[1]Area of Histology, Faculty of Medicine and INCYL, University of Valladolid, Valladolid, Spain; [2]Department of Pathology, Faculty of Medicine and Odontology, University of Valencia, Incliva, Ciberonc, Valencia, Spain; [3]Department of Cytology and Normal and Pathologic Histology, Faculty of Medicine, University of Seville, Seville, Spain; [4]Depatment of Histology, Faculty of Medicine, University of Granada, Granada, Spain; [5]Department of Cell Biology and Histology, School of Medicine, University of Murcia and IMIB-Arrixaca, Murcia, Spain;

In the opening session of the Histological Society of Madrid, founded by Maestre de San Juan and seed of SEHIT, Maestre, as president of the new society, gave the speech “Which may be the importance of Histology and the need of its study”. 148 years later, we revise the same question aiming at the current state of Histology. Histology is the basis of Physiology and Histopathology and over time it has become more and more functional and even therapeutic as part of tissue engineering, as well as multimediatic. Extraordinary possibilities have emerged thank to ICTs and LKTs both for research and teaching, albeit this virtualization is not risk free, and would we reach the case in which researchers, students and professor will only meet in a metaverse?

A bibliometric analysis of the publications confirms a consolidation of the evolution of Histology towards a constructivist paradigm inside tissue engineering, what justifies the inclusion of this subject in the programs of Histology both at the undergraduate and graduate levels. The bibliometric analysis also allows for the description of an emerging scientific language (conceptual frame) and new developing and collaborative focusses in research (social frame), as well as for the evaluation of the real impact of the area of Histology in XXI century.

Microscopic image analysis is one part of the mentioned technologies, digital pathology, which influences the most on 4P Medicine and has brought a renewal of structural and functional histological concepts. Tissue biotensegrity (tension+integrity) depends on both intracellular and extracellular matrix arrangement of forces, controlling processes such as mechanotransduction and cell shape, movement, proliferation and differentiation, among others. A deeper knowledge of this topic promises significant improvements in medicine, especially in tissue engineering.

ACADEMIA accreditation program of ANECA for University professorship has become a milestone in academic life in Spain. After its 2017 reform, it is divided into 21 categories (areas) that are grouped into 5 macro-areas. The category Histology is placed in area B6 (Biomedical Sciences) within the Health Sciences macro-area, sharing sometimes teaching and research profiles with other related areas, which give rise to overlaps and difficulties in the evaluation as well as in marking the limits to define a specific profile and criteria for the area of Histology, a matter that is crucial for applicants.

The new technologies have also highly changed the world of scientific journals. With the change of century, digital and open access journals have replaced the printed journals that charge the reader. This revolution has led to a situation in which now the one who pays is the author. In this context, it is very simple to edit a journal with some computing knowledge, and therefore scientific journal have proliferated greatly. So-called predatory journals have appeared with the only objective of making money, thus not conducting peer reviews. The computing platforms also allow for the appearance of “paper mills”, that are factories which prepare articles invented by a computer to sell them to researches who commit this fraud to deal with the publish or perish pressure. Histology journals in last century were few, but nowadays the number is much greater, including journals with very similar names, so caution must be taken by researchers to submit the article to the right journal. Scimago Journal Rank and Scopus include the category Histology, but this category was removed from the Web of Science, so it must be replaced in this listing.

Presentations:

Noguera R. “Avances conceptuales en histología”

Fernández Santos J.M. “Situación del área de histología en el programa de acreditación del profesorado de ANECA”

Martín-Piedra M.A. “Publicaciones en histología. Un análisis bibliométrico”

Madrid J.F. “Revistas científicas en el área de histología”

SESSION

EDUCATION AND TEACHING INNOVATION IN HISTOLOGY

DOCENCIA E INNOVACIÓN DOCENTE EN HISTOLOGÍA

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Relationship between study time and marks obtained

Sáez F.J. and Badiola I.

Department of Cell Biology and Histology, School of Medicine and Nursing, University of the Basque Country UPV/EHU, Bº Sarriena s/n, 48940 Leioa (Vizcaya), Spain

Studies on the relationship between the time spent by students studying and their academic performance have shown contradictory results, with some showing a positive relationship [1,2] and others a negative one [3,4]. For this reason, the lecturers of the subject Human Histology in the 1st year of Dentistry at the UPV/EHU have carried out an analysis to try to provide more data on this subject.

During the 2020-21 and 2021-22 academic years, the subject Human Histology in the first year of dentistry at the UPV/EHU was taught using the flipped classroom method and continuous assessment was carried out. Students were asked to complete a weekly survey on the eGela platform (Moodle) to indicate the time spent studying. The correlation between the final mark obtained by each student in the subject and the time spent studying was analysed. The analysis was carried out using Microsoft® Excel.

Sixty-three students followed the subject in the two academic years. The average mark obtained by the students was 7.8 out of a possible 10 points, with a maximum mark of 9.3 and a minimum mark of 5.8. The average time spent studying the subject was 123.3 hours, with a maximum value of 305.3 and a minimum value of 42 hours. The correlation coefficient between the number of hours of study and the mark obtained was -0.14. The correlation between the two variables can be seen in figure 1.

This means that there is a very weak negative correlation between the two variables. Consequently, it should be thought that the performance of each student depends more on his or her ability, study strategies, or other variables that are difficult to examine [5,6].

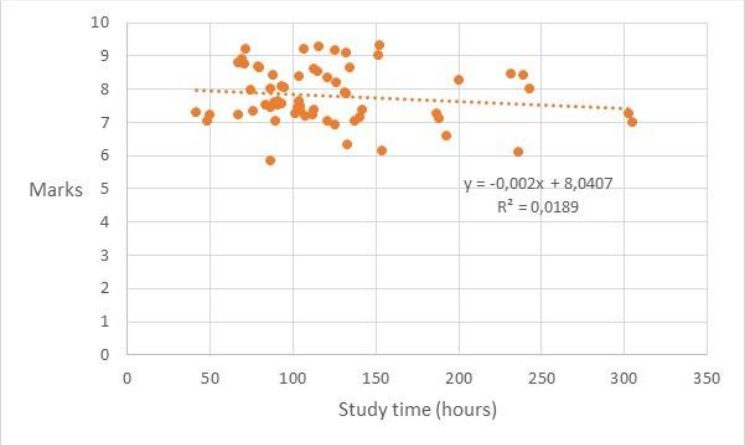


Fig. 1. Correlation between the marks obtained by the students and the hours spent studying.

Supported by an educational innovation project of the SAE/HELAZ.

[1] Stinebrickner R. and Stinebricker T.R. (2004). Time-use and college outcomes. J. Econom. 121, 243-269.
[2] Lahmers A.G. and Zulauf C.R. (2000). Factors associated with Academic Time Use and Academic Performance of College Students: A Recursive Approach. J. Coll. Stud. Dev. 41, 544-556.
[3] Krohn G.A. and O'Connor C.M. (2005). Student Effort and Performance over the Semester. J. Econ. Educ. 36, 3-29.
[4] Purdie N. and Hattle J. (1999). The relationship between study skills and learning outcomes: A meta-analysis. Aust. J. Educ. 43, 72-86.
[5] Nonis S.A and Hudson G.I. (2010). Performance of College Students: Impact of Study Time and Study Habits. J. Educ. Business 85, 229-238.
[6] Barrera Hernández L.F., Vales García J.J., Sotelo-Castillo M.A., Ramos-Estrada D.Y. and Ocaña-Zúñiga J. (2020). Cognitive Variables of University Students: their Relationship with Dedication to Study and Academic Performance. Psicumex 10, 61-74.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Histology as a backbone discipline to introduce the bachelor's degree in Medicine to high school students: The case of Practical Activities of the University of the Basque Country (UPV/EHU)

Ibarretxe G.[1] and Apraiz A.[1]

[1] Department of Cell Biology and Histology, University of the Basque Country (UPV/EHU), Leioa, Spain

Introduction: The University of the Basque Country (UPV/EHU) is the principal institution offering bachelor's degree in Medicine studies in the Autonomous Community of the Basque Country, with about 340 new students enrolled in the Medicine degree program each year. With a history of more than 50 years, and five associated University Hospitals, the Medicine and Nursing Faculty of UPV/EHU has positioned itself as a centre of reference to study Medicine in the north of Spain. Due to its public nature and the high demand for enrollment, admission to the degree in Medicine in UPV/EHU is governed by very strict academic criteria, with minimum admission marks approaching 13 points out of 14, in recent years [**1**]. In this context, there is also a high demand of information on the part of high school students about the Medicine Faculty, the medical career, and the type of practical activities that are carried out during the 6-year Medicine degree in UPV/EHU. The Vice-Rectorate for Students and Employability organizes an annual call for Practical Activities for students wishing to enroll in university studies in UPV/EHU [**2**]. However, this initiative can sometimes be difficult to implement in the case of Medicine and Nursing Schools, where many of the practical activities are carried out in a hospital care setting. In view of that, two teachers of the Medicine Faculty decided to fill in the participation void existing at that time, and applied to the call of Practical Activities in 2017, bringing a proposal for a combined activity in the fields of Cell Biology, Histology and Histopathology.
Methods: We designed a Practical Activity adapted to high-school students, consisting of a guided observation of microscopy samples, where students had to discuss in groups and perform autonomous information searches, both online and in Histology atlas books, to identify a normal tissue biopsy and compare it with a pathological one, drawing conclusions about presumptive patient diagnosis and treatment. The activity lasts 2 hours, and underscores the importance of Histology to obtain information about the normal structure of tissues, as a basis for understanding how this is altered in human diseases. The activity was complemented with an explanation about the general Medicine curriculum and the rest of studies in the Medicine Faculty of UPV/EHU, and has been carried out annually and uninterruptedly since 2017, with the sole exception of the 2020/2021 year, where it was suspended due to the covid19 pandemic.
Results: The activity was very well received by the students, who made more than 800 applications for only 48 places in the starting 2017/18 academic year. In the following two years, the number of available places rose to 250 and 150 respectively, where 82% of places were on average covered by women, and 18% by men. After its suspension in 2020/2021, enrollment in the activity was reopened again in the 2021/2022 academic year, with 88 places for 360 applicants. The activity was highly appreciated by the participants, with overall satisfaction scores of 4.63/5. Analysis of the student responses showed that most participants (80%) were happy with the duration of the activity, whereas 18% deemed it too short, and 2% too long. In view of the success, we intend to continue offering the activity for the next years, with a further increase in the number of places to try to better meet the demand from high-school students.
Discussion & conclusions: Our experience suggests that Histology is an attractive discipline to awaken and consolidate vocations in the field of Medicine. It constitutes a natural backbone linking basic science disciplines (cell biology, genetics) to medical disciplines like clinical and medical pathology. Students feel motivated and perform well when they are instructed to use a microscope to focus an unknown histological sample with their own hands, and enjoy the challenge to interpret these histological images to formulate clinically relevant conclusions.

Supported by the Vice-Rectorate of Students and Employability of UPV/EHU, and the Education Department of the Basque Government

[**1**] Transparency portal: Access to UPV/EHU. https://www.ehu.eus/es/web/sarrera-acceso/notas-corte
[**2**] University Counseling Service. https://www.ehu.eus/es/web/sou/actividades-practicas

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Effect of the COVID-19 pandemic on Histology grades

Hilario E.[1], Álvarez-Díaz A.[1], Alonso-Varona A.[1], Rodríguez-Andrés C.[1,2] and Alonso-Alconada D.[1]

[1] Department of Cell Biology and Histology and [2] Department of Preventive Medicine and Public Health, School of Medicine and Nursing, University of Basque Country. Leioa. Spain

Introduction: As a consequence of the COVID-19 pandemic, teaching became 100% virtual, not only the lectures and practices, but also the evaluation process. We wanted to unravel the possible differences in grades between the two subjects in which we teach Histology: Basic Medical Histology (BMH, 1st semester, second year) and Special Medical Histology (SMH, 2nd semester, second year). We also wanted to focus on the teaching modalities from the point of view of the qualifications obtained by the student.

Material & methods: BMH covers the topics of Tissues, Hematopoietic, Cardiovascular and Immune and Nervous System, whereas SMH includes the rest of the topics. The teaching process is carried out through theoretical lectures, classroom practices and laboratory practices (microscope with slides). Both subjects are taught to two groups of students by the same faculty, with the same credits, teaching methods and evaluation system. The final grade is a weighted average of those three modalities, in addition to the practical notebook grade prepared by the student. Both subjects are independent, and it is not necessary to have passed BMH in order to pass SMH. The grades of four consecutive academic years were collected: BMH (2018-19 to 2021-22) and three courses of SMH (2018-19 to 2020-21, since the exam for 2021-22 was not taken yet). We considered the grades of the theoretical exam, classroom practices, laboratory practices (practical exam) and the practical notebook grade. The statistical methods used were: 95% confidence interval for the means; contrast of means between genders using Student's t test; comparison of the means of the scores obtained between the different courses using one-factor analysis of variance; and study of the influence of different factors on failing the course using logistic regression models.

Results: The grades obtained by 860 students in the subject of BMH (639 women and 221 men) and by 616 students in SMH (446 women and 170 men) were collected. In BMH, the mean grade for all courses and students were as follows: theoretical exam 5.37; practical exam 7.93; classroom practice 7.37; notebook 8.74. There were significant differences in the means of the grades among the different academic courses, modalities and genders ($p<0.0044$). In BMH, no significant differences were observed in the number of failures between groups 01 and 02, adjusted by course and gender. In the 2019-20 course, BMH showed lower averages in the theoretical exam ($p<0.0001$) than those from other courses, and the number of failures doubled those of the previous one, adjusting the comparison by gender. In SMH, the mean grades for all courses and students were: theoretical exam 6.99; practical exam 8.24; classroom practice 7.34; notebook 8.86. There were differences in the means of the grades between the different academic courses and modalities ($p<0.0223$), existing, in addition, a difference in the number of failures between groups 01 and 02. In the 2019-20 course, SMH showed higher-grade means in the theoretical exam ($p<0.0001$) than those from other courses.

Discussion & conclusions: Regarding the course affected by the COVID-19 pandemic (academic year 2019-20), it should be noted that the students took BMH (first term) in the traditional way, while those same students were affected by taking SMH subject (second term) with initially face-to-face teaching, then virtual and finally 100% on-line exams. While the grades obtained in 2019-20 for BMH were very low (with a high number of failures) compared to other courses, their grades increased significantly in SMH, being much higher than those of the other courses analyzed. It is evident that an on-line exam in which the control of the test cannot be reliably accredited had an impact on the students' grades.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Creating histology comic strips using Greco-Latin etymology as an innovative teaching approach

Monferrer Garzarán E.[1,2], Navarro Noguera A.[4], Movellán Luis M.[3], Morenilla Talens C.[3], Martín de Llano J.J.[1], Ruiz Sauri A.[1] and Noguera Salvá R.[1,2]

[1] Department of Pathology, Medical School, University of Valencia-INCLIVA Biomedical Health Research Institute, 46010 Valencia, Spain; [2] Low Prevalenc Tumors, Centro de Investigación Biomédica En Red de Cáncer (CIBERONC), Instituto de Salud Carlos III, 28029 Madrid, Spain; [3] Classical Philology Department, Faculty of Philology, Translation and Communication, University of Valencia, 46010 Valencia, Spain; [4] Classical Philology Department, National University of Distance Education (UNED), Spain

Introduction: The linguistic legacy of classical languages in the scientific-medical vocabulary is undeniable, but the teaching of Greco-Latin etymology is usually insufficient in the Medicine degree. In the Classical Philology degree, however, the learning of specific terminology applied in medicine is relegated exclusively to the theoretical framework. Fostering bidirectional knowledge of these two disciplines could facilitate the assimilation of scientific vocabulary, even more so if lateral thinking is promoted through the design of comic strips or vignettes. This project aims to present basic contents of Medicine and Classical Philology in a dynamic and entertaining way to favor the autonomous learning of students.

Methods: This teaching innovation project consisted of two work modules: 1) Provide free access to teaching material for students in the first two courses of Medicine and Classical Philology. Representative microscopic histological slides of different tissues were selected. A corpus of scientific-medical terms related to the histological slides was then elaborated. Finally, graphic support material was designed through the elaboration of vignettes of caricatured histological images. Self-evaluation of the students was carried out through a test in the virtual classroom. 2) Voluntary theoretical-practical workshops on eloquent images and ICTs (Information and Communication Technologies). The discussion on new histological images and the etymological explanation of the selected terms was moderated. After explaining digital tools for the search for Greco-Latin terminology, vignette design and image repositories, the students created a virtual comic strip in situ of a histological image for autonomous work and subsequent self-evaluation.

Results: The participation of the students was disparate, highlighting the particular interests of each field of knowledge. In Classical Philology, 45% of the students consulted the vocabulary, 69% viewed the video and 63% carried out the self-assessment. In Medicine, only 26% of the students consulted the vocabulary, 14% viewed the video and 12% performed the self-assessment. Remarkably, the participation of first-year Medicine students was greater than that of second-year students. In addition, a greater participation of the groups of students that were motivated by the teaching staff was observed. Regarding the theoretical-practical workshop, 33 students of Classical Philology participated, but only 2 of Medicine.

Discussion & conclusions: These results suggest that Classical Philology students accepted better the novel teaching methodologies presented. Interestingly, Classical Philology students were more interested in playful activities (video and workshop), while the medical students preferred the theoretical content presented in the vocabulary. Overall, this project reveals the specific needs of the students, which will be integrated in the future, and highlights the importance of the teacher in the students' motivation.

Supported by SFPIE (Servei de Formació Permanent i Innovació Educativa), University of Valencia, Project ID: UV/SFPIE_PID-1641957.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Development and design of a virtual and interactive tool for teaching of Medical Histology

Jimena I.[1,2], Tarradas-Merino E.[3], Agüera A.[1], Aldebis A.M.[1,2], Cantarero I.[1] Moreno-Llorente D.C[1]., Martín A[1]. and Luque E.[1,2]

[1] Department of Morphological Sciences, Section of Histology, Faculty of Medicine and Nursing, University of Córdoba, Spain, [2] Maimonides Institute for Biomedical Research IMIBIC, Reina Sofia University Hospital, University of Córdoba, Spain, [3] Teaching and Research Support Service (SIADI), Faculty of Medicine and Nursing, University of Córdoba

Histology is a very visual-oriented subject whose teaching and learning is based on the observation of microscopic images.
In order to make the teaching and learning process easier, especially during the current pandemic situation, we have created a virtual and interactive tool through the Genially software in which we have created visual and interactive content. Later, we will upload this tool into the Moodle learning platform so that students can use it.
By observing the histological samples uploaded into this tool, which will be similar to those studied in the Microscopy Classroom and previously discussed during the practice sessions, the students will be able to identify and analyse their different structures (Fig. 1).

Figure 1. Histological sample of the oesophagus. All the commands that can be accessed are displayed.

The advantage of this kind of tool is that it is not limited in time or space for the students, and it allows them to complete the practice sessions which are taught in the laboratory and in the Microscopy Classroom by the teaching staff of the Histology Area at the Faculty of Medicine and Nursing of the UCO.
Furthermore, it allows a medical approach to the subject, since the histological samples have been created from human samples and they are the same or like those that have been displayed in the practice room. We also have included several staining techniques which are necessary for the right visualization of a certain structure.
It allows interaction on active areas or through icons to choose the observation of a certain area and to visually analyse the different tissues and structures that form it. It also gives the possibility of following its description through oral or written locution according to students choice.
The tool incorporates a self-assessment system in which the student, faced with normal or pathological images, will have to choose the one that corresponds to the indicated structure, or even differentiate the normal structure between several pathological structures.
In conclusion, we believe that it is a suitable tool to complement the face-to-face teaching in Medical Histology subjects given its simple use, interactivity, medical-oriented content, and self-assessment options.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Program of the teaching-learning unit anatomy and histology of mammals with an eclectic didactic approach

García-Lorenzana M.[1], Beltrán-Vargas N.E.[2] and García-Lorenzana O.[3]

[1] Área de Neurociencias, Departamento de Biología de la Reproducción, Universidad Autónoma Metropolitana-Iztapalapa, CDMX, México; [2] Departamento de Procesos y Tecnología, Universidad Autónoma Metropolitana-Cuajimalpa, CDMX, México; [3] Asesor independiente, San Luis Potosí, México.

At the Autonomous Metropolitan University (UAM) the academic units that make up the study plans of the different undergraduate or postgraduate degrees are called teaching-learning units (TLU). The TLU's that deals with the study of the normal and pathological morphophysiology of the organic systems of vertebrates is affected by factors: a) students' own, b) of Anatomy and Histology c) institutional. Obviously, the above factors are impacted by the current health conditions that impose the need for a program in the hybrid mixed modality (face-to-face-virtual).
With the aim of facing the problem arising from the interrelation of the factors exposed above, we consider that it can be solved based on the implementation of a different didactic that seeks: 1) to start from the consideration of socioeconomic factors that affect students, 2) make efficient use of individual and institutional time as well as material and human resources, 3) design instruments that facilitate the use of the terminology of Anatomy and Histology, 4) carry out activities and adopt techniques and teaching materials that promote the development of forms of reasoning and analysis that allow the construction of knowledge and its application in real situations, typical of Anatomy and Histology, 5) develop teaching resources that allow the efficient use of information and communication technologies [1].
In this sense, we have implemented activities based on the principles of complex thinking. For this we consider the articles that in a timeline conclude with the concept of nerve growth factor coined by Drs. Rita Levi-Montalcini and Stanley Cohen, with whom they obtained the Nobel Prize in 1986. The objectives set out in this exercise seek to confront students with the approach of questions, hypotheses, methodology, techniques, and reasoning that allow them to interpret the experimental results obtained by research, with ingenuity, creativity, and willpower, as Drs. Montalcini and Cohen, who managed to overcome the adverse conditions of the social and economic environment typical of the Second World War. For this, a complex didactic strategy was developed that involves several moments in which collaborative, constructivist and competency-based learning techniques are applied, both face-to-face and virtual, which guide the key theoretical, methodological, and technical concepts of each article as well as the impact that they have in regenerative and/or translational medicine.
There were 2 groups of students, and this methodology was applied to one of them, obtaining better results than the traditional group. To evaluate the results of the exercise, an open questionnaire was considered that had scores of 82% "very good", and 18% "good"; while in the control group 42% "very good", and 58% "good".

[1] García-Lorenzana, M. 2002. Un escenario posible en la enseñanza de la morfofisiología animal. En Nuevos Retos de la Docencia e Investigación en Histología. SMH-UAMI.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Histology for everyone: the UVigo histological atlas

Megías M., Molist P. and Pombal M.A.

Departamento de Bioloxía Funcional e Ciencias da Saúde, Facultade de Bioloxía-IBIV, Grupo Neurolam, Universidade de Vigo, 36310 Vigo, Spain.

The histology atlas of the University of Vigo was initiated within the framework of the Bologna plan, which encompasses new teaching methods to promote and improve the autonomous work of students. In this sense, we wanted to offer our students a tool to successfully complete their training. Our goal was to create an interactive Internet site dedicated to plant and animal cytology, histology and organography. The URL is http://webs.uvigo.es/mmegias/inicio.html and it was constructed using open-source software.

Our main goal was to provide texts and images that can help students and other people interested in these subjects. More specifically, the objectives are:

a) Enhance the learning of plant and animal histology through a complete and integrated set of contents.

b) Improve skills to identify tissues and cell structures. It is important to observe cell and tissue structures to gain a solid foundation in histology. This will be a major point of this site. Therefore, we select high-quality images to show outstanding morphological features of cells, tissues, and organs. In addition, there are interactive pages and quizzes designed to assess learning progress.

c) Cover basic and advanced knowledge of cell biology and histology. All sections have a similar structure. First of all, there is a set of pages with basic and essential information. From this level it is possible to jump to other pages where more detailed information is provided. Furthermore, images for training are linked to both basic and advanced pages.

The Histological Atlas is organized in sequentially ordered sections to facilitate learning; however, each of them can be consulted independently, without following the suggested sequence. The sections are as follows: cell, cell types, plant tissues, animal tissues, plant organs, animal organs, histological techniques, virtual microscopy, downloads, news, index, presentation and also an acknowledgments section.

In September it is the 15th anniversary of the first uploaded pages of this project. The content of the Histological Atlas has been growing from its beginnings to the present with the constant incorporation of new content, texts and graphic material, such as: summaries, images, quizzes, and new sections. These are announced on the news page. Our intention is to continuously expand and improve the site. It is also open to collaboration, so we encourage anyone interested to contribute content or suggestions. Now, most of the site is also available in English. Since September 2007, the site has received more than 25.797.519 visitors from all over the world, who visited 79.506.986 pages, being the record number of visitors in a single day 40000 in October 2021.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Approach histopathology beyond medicine. A transversal teaching innovation project

Leiva-Cepas F.[1,2], Osuna-Soto J.[2], Galvez Medina M.J.[2], Sanchez Ramirez I.[2] and Moreno Gutiérrez J.A.[3]

[1] Department of Morphological and Sociosanitary Sciences. Faculty of Medicine and Nursing. University of Cordoba. Cordoba, Spain; [2] Pathology Unit. Reina Sofia University Hospital. Cordoba, Spain. [3] Department of Cell Biology, Physiology and Immunology. Science Faculty. University of Cordoba. Cordoba, Spain.

Introduction: The practical teaching of related basic disciplines such as Histology and Cell Biology, which are eminently visual, together with their medical application in the case of Pathology, leads us to integrate in students the alterations of histological normality as a basis for knowledge of the different pathologies [1,2]. The main objective is to know the degree of satisfaction and the usefulness of an innovative practice in a non-medical subject through a regulated collaboration with a Pathology Unit.

Methods: 48 students of the Biology degree carried out a practice of visualizing digitized human biopsies, available on the virtual platform with free access for students; They were divided into groups. Each group was assigned images of a pathology selected according to its cellular alteration and corresponding pathology. The students labeled the images and made a summary with explanatory texts that helped them learn the concepts under study. On each problem image, the students identified the tissue/organ, the "residual" histological elements or structures and their alterations and identified all the structures to label them in the digital image. In addition, the students searched the literature to relate normality to abnormality. Finally, they defended their hypotheses about tissue alterations and their relationship with the histopathological lesion. This activity was evaluated by an anonymous survey that collected 12 items: general questions (2 items), objectives, methodology and motivation (4 items), evaluation of the practical seminars (4 items) and general evaluation of the subject (2 items). Each item was evaluated on a Likert scale from 0 to 5, except the last two, which were grouped into: excellent, acceptable, fair and poor. A descriptive analysis of the items addressed in the study was performed. The results obtained between the items within each methodology were compared using the Student's t-test.

Results: The survey was answered voluntarily by 85.48% of those enrolled in the subject (24 women -58.5%-, 16 men -2.4%- and 1 person without assigned gender -2.4%-). The responses were grouped into 3 blocks: 1) evaluation of the methodology used with a mean score of 4.31; 2) usefulness of the activity, which obtained a mean score of 4.41; 3) general evaluation of the innovative teaching practice where 53.6% -22- had an excellent opinion of it, 43.9% acceptable -18- and 2.5% regular -1- (Fig 1.).

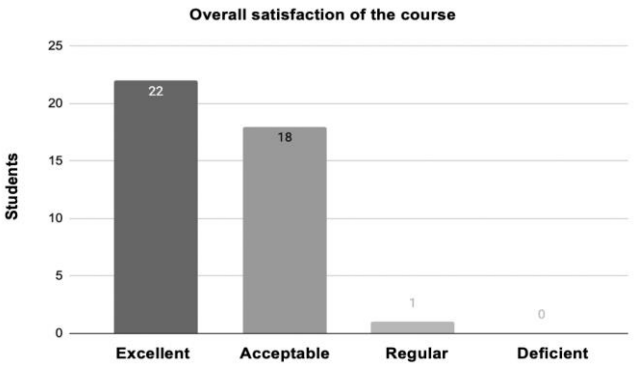


Fig 1. Percentage of overall student satisfaction with the subject.

Discussion & conclusions: The interpretation of histopathological images has been a teaching innovation highly valued by the students. Teaching strategies must be implemented in different subjects of the study plans to promote adequate interaction between health and non-health professionals as a projection to a more transversal university teaching.

[1] Danks JA, *et al*. *J Histotechnol.* 34:119-123. 2013.
[2] Kumar RK, *et al*. *Anat Rec B New Anat*. 289(4):128-133. 2006.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Are the students of General Histology from the Degree in Medicine satisfied with the practical activities in this subject?

Ruiz-Saurí A.[1,2], Martín de Llano J.J.[1,2], Mata M.[1,2], Noguera R.[1,2], Sancho-Tello M.[1,2], Gómez R.[1,2], Milian L.[1,2], Molina P.[1,3], Sepúlveda P.[1,4], Ríos-Navarro C.[1,2], Vieco I.[1,2] and Carda C.[1,2]

[1] Department of Pathology, Faculty of Medicine, Universitat de Valencia, Valencia, Spain; [2] Institute of Health Research-INCLIVA. Valencia, Spain; [3]Forensic Pathology Service, Institute of Legal Medicine and Forensic Sciences, Valencia, Spain; [4]Instituto de Investigación Sanitaria La Fe, Valencia, Spain.

Introduction: At the Faculty of Medicine and Dentistry from the University of Valencia, a total of 320 students were enrolled for the first time in the subject General Histology at the Degree in Medicine. In this subject, 60% of the learning hours are related to theory and 40% to practice. The practical contents of General Histology are divided into three main activities: i) 30 histological pictures to diagnose it and identify the type of staining; ii) 22 slices from different human tissues to visualized them under light microscopy; iii) 9 seminar sessions prepared and exposed in groups of 4 to 5 students based on an specific topic assigned at the beginning of the term. In order to improve the teaching quality of our university, an anonymous survey regarding the opinion about the practical activities taken place at the subject General Histology has been done to all the students enrolled at this subject.

Materials and methods: Table 1 displays the survey prepared by the teaching staff and anonymously answered by all the students enrolled at General Histology during the last practical session.

Results: A total of 301 (out of 320 students) surveys have been completed and the data are shown in Table 1.

Conclusion: In the survey answered by the students enrolled in the subject General Histology at the Degree in Medicine and related to their satisfaction about the practical sessions of the subject, we conclude that students prepare working with microscopic sessions and histological pictures, but they found useful for their future career to prepare and expose seminars.

Questions	Percentage of students (%)		
	Microscopy Sessions	**Seminar Sessions**	**Histological pictures**
Which is the most interesting practical activity?	77.93	2.34	19.73
Which is the practical activity from which you learnt the most?	60.19	1.29	38.51
Question	**Preparation**	**Exposition**	**Team work**
Which is the most interesting part of the seminar sessions?	28.99	30.43	40.58

Questions	Percentage of students (%)	
	Yes	**No**
Would you increase the number of microscopy sessions?	41.69	58.31
Regarding the seminar sessions, do you think they are useful to further comprehend the subject?	30.51	69.49
Do you think the current way of working in the seminar sessions are useful for future career?	14.19	85.81
Do you think preparing presentations for the seminar sessions are useful for your future career?	68.71	31.29
Would you change the seminar sessions for a higher number of microscopy sessions?	70.07	29.93
Would you change the seminar sessions for a higher number of histological pictures?	62.24	37.76

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Evaluation of seminars with integrated clinical and histological imaging as a learning tool in medical histology

Chato-Astrain J.[1,2], Martín-Piedra M.A.[1,2], Ortiz-Arrabal O.[1,2], Sánchez-Porras D.[1,2], García-García O.D.[1,2], Oyonarte S.[1,2,3], Carriel V.[1,2], Campos F.[1,2] and Rodríguez I.A.[1,4]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Network for Transfusional Medicine, Cells and Tissues, Granada, Spain; [4] Cátedra "B" de Histología y Embriología, Facultad de Odontología, Universidad Nacional de Córdoba, República Argentina

Introduction: The seminar has been used for years in the complementary formation of Medical Histology with variable results in terms of achieving the proposed objectives [1]. In this work, we implemented a teaching program based on the analysis and discussion of a clinical case based on histological images that are analyzed with the students. The objective of this program is to promote the active participation of the students and the interaction with the teachers towards a correct interpretation of the data to establish a potential histological diagnosis.
Materials and Methods: After the program was implanted, students were surveyed by using a questionnaire designed ad hoc for this purpose. 90 students enrolled in the subject of Histology at the Faculty of Medicine at the University of Granada were included in the study. Three levels were evaluated in the questionnaire: 1) the communication capabilities of the teacher and the didactic resources used, 2) the previous preparation of the students and 3) the improvement of interpretation and integration of the histological preparations. Each item was evaluated using a Likert-like scale with four categories: 1 (very good), 2 (good), 3 (fair) and 4 (poor).
Results: Students rated levels 1 and 2 as very good in more than 80% of the cases. However, level 3 was rated very low, with most students rating this level as good or fair (Figure 1).

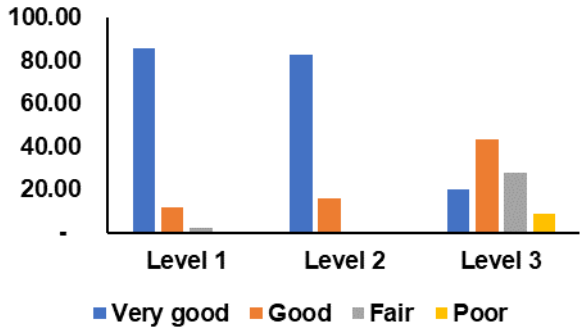


Fig. 1. Percentage assigned by the students to each level of question.

Discussion & Conclusions: The use of this didactic modality based on the discussion of clinical cases in seminar sessions with clinical and histological images was very positively evaluated. Students rated very positively the communication capabilities of the teacher and the didactic resources used during the session, and were aware of their previous preparation and study to the seminar. These results suggest that this didactic approach based on the integration of clinical and histological data is very well valuated by the students and promotes the student preparation for the session.

This work was supported by Proyecto de Innovación y Buenas Prácticas Docentes, Universidad de Granada, plan FIDO: 20-105.

[1] Campos A, et al. Histol Med. 1985;1:127-134

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Differences in health sciences students perception of threshold concepts linked to tissue engineering

Martin-Piedra M.A.[1,2], Saavedra-Casado S.[3], Santisteban-Espejo A.[4], Blanco-Elices C.[1,2], Fernández-Valadés R.[1,2,5], Sola M.[1,2], Ruyffelaert A.[6], Garzón I.[1,2] and Campos A.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Spain; [3] PhD Program in Biomedicine, University of Granada, Spain; [4] Department of Pathology, Puerta del Mar University Hospital, Cádiz, Spain; [5] Division of Pediatric Surgery, University Hospital Virgen de las Nieves, Granada, Spain; [6] Department of French Philology, University of Granada, Spain

Introduction: Since its introduction, in 2003, the threshold concepts (TC) theory has attracted the attention of the scientific community. This pedagogical theory hypothesizes the existence of several concepts whose comprehension resemble conceptual gateways or portals that lead to a previously inaccessible way of thinking about something [1]. In this sense, TC have also been proposed as a relevant tool for a focused curricular redesign as the teaching of these notions may significantly improve the students' learning about crucial aspects of each discipline [2] and particularly in medical subjects, including the Human Histology [3]. The aim of this study is to identify TC related to tissue engineering on undergraduate students of different health sciences curricula through the analysis of the students' perception.

Methods: A total of 410 students enrolled in the subject of Human Histology participated in this study. 244 students corresponded to the degree in Medicine, 64 to the degree in Dentistry and 102 students were enrolled in the degree in Pharmacy. Perceptions of TC linked to tissue engineering were evaluated using a validated and registered questionnaire. Specifically, the concepts of "native tissue", "artificial tissue" and "cell, tissue and organ culture" were assessed as TC. The survey was performed once the subject teaching was finished, and students were instructed on TC nature and characteristics. Two-way ANOVA was used to compare the results among the three groups. Effect sizes of the differences were calculated as Cohen's d (Δ).

Results: Perceptions of undergraduate students of tissue engineering concepts showed remarkable differences among groups (Figure 1). Dentistry students valued these concepts with highest scores (4.1 ± 0.9), being these values significantly higher than those of medical students (3.7 ± 1.0, $p < 0.001$) and pharmacy students (3.6 ± 1.1, $p = 0.007$). Also, the effect size for the difference in the perception of tissue engineering concepts showed differences between dentistry and pharmacy students (Δ = 0.436), revealing that this difference is not only significant, but also remarkable.

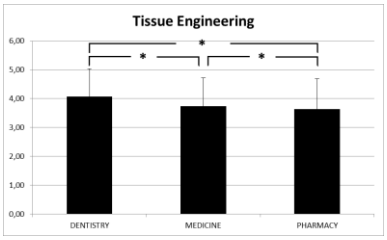


Fig. 1: Students' perceptions of different concepts linked to tissue engineering as threshold

Discussion & conclusions: These results suggest that undergraduate health sciences students feel some differences in the perception of some concepts linked to tissue engineering as TC. Medicine and Dentistry students perceived these concepts with more intensity than pharmacy students and these results may be explained by the different conception of their own degrees. In medicine and dentistry degree programs, Histology is conceived as a discipline focused on the treatment of human diseases with artificial tissues as substitutes, through the application of tissue engineering. However, pharmacy students perceive the comprehension of histological structures, as liver or kidneys, for further comprehension of drug metabolism and possible drug effects associated to these structures. The results of this study reveal the need to include tissue engineering concepts on the teaching of Histology, as well as the application of different strategies optimized to the specific perceptions of students in each health science curriculum.

Supported by CTS-115 (Tissue Engineering Group).

[1] Meyer J, Land R. High Educ. 2005;49:373-88
[2] Entwistle N. Threshold Concepts within the disciplines. 2008; Sense Publishers
[3] Santisteban-Espejo a, et al. Med Hypotheses. 2020;143:110099

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Implementation and evaluation of a practical model oriented to the acquisition of clinical competences in Medical Histology

Sánchez-Porras D.[1,2], Chato-Astrain J.[1,2], Sola M.[1], Blanco-Elices C.[1,2], González Quevedo D.[3], Santisteban Espejo A.[4,5], Ortiz-Arrabal O.[1,2], García-García O.D.[1,2] and Campos F.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Department of Orthopedic Surgery and Traumatology, Regional University Hospital of Málaga, Málaga, Spain; [4] Department of Pathology, Puerta del Mar University Hospital, Cádiz, Spain; [5] Institute of Research and Innovation in Biomedical Sciences of the Province of Cadiz (INiBICA), University of Cádiz, Cádiz, Spain

Introduction: Microscopic observation of histological preparations is a very valuable tool for the acquisition of transversal competences in practical teaching in Histology [1]. Specifically, acquisition of relevant skills related to the observation, identification and diagnosis of histological preparations could contribute to develop essential basic competences by the students, which will be the basis for their future clinical practice. The purpose of the present study is to evaluate the competences reached by medical students enrolled in the Histology program to identify and diagnose relevant structures that are observed microscopically, and to assess how the students improve their skills in Histology with the development of sequential practical sessions.
Methods: Four practical sessions related to the histological structure of different types of human tissues were evaluated (the first two sessions and the last two sessions of the practical program of the Medical Histology subject). In all cases, 90 students who had previously received theoretical teaching on these topics, were asked to answer to a questionnaire designed *ad hoc* for the present study. This questionnaire consisted of several items related to the identification of the structures shown in the practical sessions (identification of three key structural elements of each histological pattern) and to the diagnosis of the tissue or organ shown in the sessions (identification of the type of tissue). Results were analyzed to compare the results obtained for the first two sessions vs. the last two sessions in order to analyze the progressive improvement of the students' skills along successive practical sessions.
Results: Results corresponding to the identification of key histological structures in the first two practical sessions revealed 53.7% of correct answers, whereas results corresponding to the last two sessions showed 63% of correct answers. Differences between the first and the last practical sessions were statistically significant ($p<0.05$). Furthermore, the data corresponding to the diagnosis of the type of tissue shown in the practical sessions varied from 33.3% of correct answers in the first two sessions to the 88.9% of correct answers obtained in the evaluation of the last two practical sessions. Differences were also statistically significant ($p<0.05$).
Discussion & conclusions: The analysis of identification competences of key elements of tissues and histological structure diagnosis confirms a progressive improvement in the acquisition of histological competences by the students during the whole period of practical sessions. We suggest the practical sessions to be organized with a clear orientation towards the acquisition of identification, observation, and diagnostic competences. These results suggest that practical sessions in Histology could contribute to enhancing important competences linked to clinical activity that the students will need to develop throughout their curricular training.

This work was supported by Proyecto de Innovación y Buenas Prácticas Docentes Universidad de Granada, plan FIDO: 20-105.

[1] Peña J. Res Novae Cordubense 2007; (4): 31-46

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Community education in advanced therapies. Investigation on the conceptual bases in tissue engineering among family medicine physicians

García-García OD.[1,2], Sola M.[3], Campos-Sánchez A.[4], Blanco-Elices C.[1,2], Ortiz Arrabal O.[1,2], España-López A.[5], Ruyffelaert A.[6], Chato-Astrain J.[1,2] and Campos F.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Family Medicine Unit, School of Medicine, University of Granada; [4] Analysis of the Andalusian Educational Reality Group HUM-672, University of Granada, Granada, Spain; [5] Craniofacial Malformations and Cleft Lip and Palate Management Unit, University Hospital Virgen de las Nieves, Granada, Spain; [6] Department of French Philology, University of Granada, Spain

Introduction: As the main gateway to the healthcare system, family medicine professionals play a crucial role in medical education. Family medicine physicians provide longitudinal, integrated, and comprehensive care to patients and, in coordination with the other levels, offer care to families and guidance to the community [1]. An important challenge for family medicine over the next decades is the so-called advanced therapies, which include gene therapy, cell therapy and tissue engineering, in the framework of regenerative medicine. The conceptual profiles of family medicine professionals regarding tissue engineering as a relevant key element in advanced therapies, were investigated to evaluate the formation received in this area in Spain.

Methods: Twenty Primary Care Health Centers belonging to the Andalusian Public Health System were enrolled in the study. A total of 94 professionals who work as family doctors in these centers were evaluated. The average age of the participating family doctors was 49.61 ± 9.55 years. 44 (46.8%) of the 94 participants were male (mean age 50.34 ± 10.09 years) and 50 (53.2%) were female (mean age 48.96 ± 9.09 years). A questionnaire was designed to specifically explore 5 conceptual components related to tissue engineering, with 6 specific items included in each component. Family medicine physicians were asked to rate their responses using a five-point Likert-type scale: 1 = strongly disagree, 2 = disagree, 3 = neither agree nor disagree, 4 = agree, and 5 = strongly agree. Mean values and standard deviations were calculated for each item. The values were assessed for all participants and for men and women, separately. ANOVA test was used to identify statistical differences.

Results & Discussion: In this work, we have investigated the tissue engineering conceptual components related to the use of advanced therapies in family medicine physicians, as we have previously done with family medicine residents [2]. There were significant differences between the conceptual components studied ($p<0.001$). For the overall topics included in the conceptual component, the topic "Cellular and tissue basis of the human body" had the highest values (3.83 ± 0.98), and the topic "Regulatory framework" had the lowest value (2.22 ± 1.13). Furthermore, significant differences between male and female specialists were only detected in the topic "New medical products", with higher values for men. Because the knowledge of the tissue engineering conceptual components is necessary to understand the meaning and role of advanced therapies in current medicine, the results of this study related to each component may be useful for the future implementation of specific education programs on the subject, which would be very positive for doctors and patients.

Supported by CTS-115 (Tissue Engineering Group).

[1] Starfield B. Lancet. 1994;344(8930):1129-33
[2] Sola M, et al. PLoS One. 2019;14(4):e0214950

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Evaluation of conceptions of learning in postgraduate students

Campos F.[1,2], España-López A.[3], Ávila-Fernández P.[1], Oyonarte S.[1,2], Ruyffelaert A.[4], Crespo P.V.[1,2], Fernández-Valadés R.[1,2,3,5], Sánchez-Quevedo M.C.[1,2] and Alaminos M.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Craniofacial Malformations and Cleft Lip and Palate Management Unit, University Hospital Virgen de las Nieves, Granada, Spain; 4 Department of French Philology, University of Granada, Spain; 5 Division of Pediatric Surgery, University Hospital Virgen de las Nieves, Granada, Spain

Introduction: Conceptions of learning refer to the beliefs and understanding that learners have about learning [1]. The relevance of these conceptions is gaining increasing interest in the learning process, as conceptions are directly related to the student's interest and motivation. Despite its importance, conceptions of learning are poorly understood, especially in medicine and health sciences.

Methods: To investigate the students' conceptions of learning, we used the previously designed and validated Learning Inventory Conception Questionnaire (COLI) [2]. This questionnaire contains several items associated to six factors related to the learning process: a) gaining information (INFO); (b) remembering, using, and understanding information (RUU); (c) a duty (DUTY); (d) personal change (PERS); (e) a process not bound by time or place (PROC) and (f) social competence (SOC). The questionnaire was used to evaluate the conceptions of learning of 131 students enrolled in four postgraduate master programs of the University of Granada, one of them corresponding to the area of medicine and health sciences, and the other three related to other sciences. Each student was asked to answer to each item using a Likert-like scale ranging from 1 (strongly disagree) to 7 (strongly agree).

Results: In general, most items were rated very highly by the students. In health science master students, the highest scores were found for the PROC factor (average 6.1±0.9), followed by the SOC factor (average 5.8±0.8), whereas the factors with the lowest valuation were RUU (5.4±0.7) and DUTY (5.5±1.0). For non-health science students, the highest values were corresponded to the PROC factor (6.3±0.8) and PERS (5.4±0.1), with the lowest scores assigned to DUTY (5.0±1.2) and INFO (5.1±1.1). Differences were statistically significant for the comparison of health science and non-health science students for the factors INFO, DUTY and SOC, which were higher in health science students.

Discussion & conclusions: These results confirm the importance that the students assign to concepts of learning, although some conceptions were rated with higher scores than others. In addition, health sciences master students had different conceptions of their own learning process than students of other types of master. The fact that health sciences students showed special consideration for the factors related to their social competence and duties could be related to the orientation of these students towards a social service to the community. In general, these findings could be useful to design future learning programs in medicine and health sciences that are more oriented to the conceptions of learning preferentially found in these students.

This study was supported by CTS-115 (Tissue Engineering Group).

[1] Säljö R. 1979. Learning in the learners' perspective. I: Some common sense conceptions. (Report No.76), Institute of Education. University of Göteborg

[2] Purdie N, Hattie J. Assessing Students' Conceptions of Learning. AJEDP. 2002;2:17-32

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Professional and motivational profiles of postgraduate master program in tissue engineering and advanced therapies applicants

Díaz-Navarro A.[1], Sánchez-Porras D.[2,3], Cárdenas-Cruz A.[4], García-García O.D.[2,3], Chato-Astrain J.[2,3], Blanco-Elices C.[2,3], Rodríguez I.A.[2,5], Ruyffelaert A.[6] and Carriel V.[2,3]

[1] Postgraduate Master Program in Tissue Engineering and Advanced Therapies, University of Granada, Granada, Spain; [2] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [3] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [4] Departamento de Medicina, Universidad de Granada, Spain; [5] Cátedra "B" de Histología y Embriología, Facultad de Odontología, Universidad Nacional de Córdoba, República Argentina; [6] Department of French Philology, University of Granada, Spain. Contact: vcarriel@ugr.es

Introduction: Tissue engineering (TE) is a multidisciplinary field focused on the development of biological substitutes to repair, replace or restore damaged tissues and organs [1]. Although previously described, this discipline was boosted up in 1993 with the article *Tissue Engineering* published by Langer & Vacanti [2]. In 1999, the Department of Histology of the University of Granada implemented the first official postgraduate master program in tissue engineering. Since then, different health science professionals, mostly medical doctors, became enrolled in this program. However, several novel University Degrees have been created over the recent years, and the interest of postgraduates of these Degrees in TE is increasing. The aim of this study is to determine the professional background and motivational profiles of postgraduate students applying for enrolment in the Master Program in Tissue Engineering and Advanced Therapies (MTEAT) of the University of Granada in order to assess the evolution of the profile of professionals interested in this discipline.

Methods: A specific questionnaire was used to determine the professional profiles of applicants to the MTEAT program. This questionnaire specifically inquired about the degree, age, gender, and motivational profiles (extrinsic and intrinsic) of the students applying for a student position in the MTEAT program in 2021. This questionnaire was filled by the applicants using an online formulary.

Results: Concerning the professional profiles of the applicants, we found a high heterogeneity, with a total of 11 degrees: 85% of the applicants were biotechnologists (25%), biologists (24%), medical doctors (20%), and biochemists (16%). Surprisingly, fewer applicants were found from health engineering (2.6%), nursing (2.6%), biomedicine (1.3%), or biomedical engineering (1.3%). When we grouped these profiles in two categories, we found that professionals with scientific/experimental profiles represented 70.65% of the applicants, while those with clinical profiles were 27.99%. Regarding the motivational profiles, the highest scores were found for 1) increase professional competence (4.68/5), 2) personal interest in the field (4.59/5); 3) improving my professional experience (4.56/5), and 4) improving my curricula (4.26/5). In contrast, less interest was found related to 1) social recognition (2.53/5); 2) achieving an economic improvement (2.99/5); and 3) redefining professional and job profiles (3.09/5).

Discussion: This preliminary study confirmed that the MTEAT program calls the interest of professionals with different graduate profiles, mostly related to scientific/experimental careers, such as biotechnology and biology, followed by medical doctors. The motivational profile analysis confirmed that applicants had a clear intrinsic motivation to the MTEAT program than extrinsic motivational factors. These preliminary findings are highly valuable as they could contribute to redefine the access criteria to our program and the didactic strategies used in teaching the program subjects.

This study was supported by Postgraduate Master Program in Tissue Engineering and Advanced Therapies of the University of Granada, Spain, and the Tissue Engineering Group (CTS-115) of the University of Granada, Spain.

[1] Campos A. Anales RANM. 2019. DOI: 10.32440/ar.2019.136.02.rev06
[2] Langer R & Vacanti J. Science. 1993; 260: 920-926

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Teaching in the subject General Structural Biophatology: Evolution of pre- and post-pandemic results

Garcia-Gallastegi P.[1], Márquez J.[1], Alonso E.[2], Jiménez L.[2] and Crende O. [2]

[1] Department of Cell Biology and Histology, Faculty of Medicine and Nursery, University of the Basque Country, Leioa 48940, Bizkaia, Spain; [2] Department of Cell Biology and Histology, Faculty of Pharmacy , University of the Basque Country, Vitoria-Gasteiz 01006, Araba, Spain.

Introduction: The subject General Structural Biophatologhy is an optional subject taught in the third year of the Degree in Pharmacy at the UPV/EHU. In the 2019/20 academic year, due to the pandemic and the state of alarm, face-to-face teaching was replaced by online teaching, through the Blackboard Collaborate platform. Given this situation, both teachers and students were vulnerable and dependent on technological resources and internet connection at home. The work relegated to the digital or e-learning field, could aggravate the technological gap and impact on the task of students and their academic results. Given the exceptional situation experienced, the teachers wanted to know the degree of satisfaction of the students, the difficulties that arose and if they were able to achieve the learning results of the subject.
Methods: At the end of the course in pandemic, a survey was designed through the virtual platform Moodle to assess the opinion of students regarding the teaching and learning received through the online modality. They were asked 12 items that were answered anonymously and voluntarily by 30 students. In addition, the academic results obtained by the students during the pre-pandemic and post-pandemic courses were compared with those obtained during the confinement.
Results: On the one hand, the survey showed that all students had a personal computer at home. In fact, 80% said they had received online teaching before. Eighty-five per cent of the students were satisfied with this modality, while only 15 per cent replied negatively. However, two thirds of the students stated that they had needed more time than usual to perform the tasks and study. In fact, some pointed out that it was harder to concentrate at home.
85% concluded that the online modality had been useful and satisfactory to achieve the competences and learning results of the subject. In addition, 70% of students indicated that the degree of knowledge acquired in the online modality corresponded to those acquired in the face-to-face modality. To facilitate the study, the students expressed that it would have been positive to have had the possibility of having the master sessions during a time in the teaching platform, where all the material that the teachers provide them is located.
A comparison of learning outcomes from pre- and post-pandemic academic courses revealed that students in confinement obtained better grades.
Discussion & conclusions: The virtual modality can support students and have online content on the platform, as well as autonomous work, improve academic results.
In this way, and in view of the general satisfaction obtained by the students, a bimodal teaching is proposed, sharing face-to-face teaching with the virtual modality. For this reason, we believe that digital competence should be developed at the University.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

The value of Kahoot® as a predictive tool for knowledge acquisition

Garza M.C.[1*], Oliván S.[1,3*], Monleón E.[1], Cisneros A.I.[1], Barrios A.[1], Ochoa I.[1,3], Whyte J.[1] and Lamiquiz-Moneo I.[1,2]

[1]Departamento de Anatomía e Histología Humanas. University of Zaragoza. Zaragoza, Spain; [2]Hospital Universitario Miguel Servet, CIBERCV, Zaragoza, Spain.; [3]TMELab, I3A_IIS Aragón. CIBER-BBN. University of Zaragoza. Zaragoza, Spain; *Both authors contributed equally

The "cultural and generational" change of our Gen Z students, the first digital natives, is supported from the Higher Education Space (EESS), embracing a commitment to a methodological renewal that improves the quality of teaching and encourages student participation and motivation in higher education classrooms. Game-Based Learning (GBL) appears as an alternative teaching methodology to achieve the improvements in the teaching-learning process suggested by the EESS. Numerous benefits of GBL, such as the use of the Kahoot® software, have already been widely described in terms of encouraging student engagement and creativity, as well as improving student motivation in dealing with different subjects. However, it has not yet been evaluated whether the Kahoot® software can be a formative evaluation tool in 2 different subjects within the Medicine career: Neuroanatomy and Histology.

Neuroanatomy group: A prospective experimental study was carried out on a sample of 173 students, belonging to two different class groups enrolled in Neuroanatomy during the 2021–2022 academic year. Prior to the final exam, 125 the students completed the Kahoot® exercise individually and identified themselves, in order to correlate this data with the results of the final exam. Final exam was conducted two weeks after the use of Kahoot® and consisted of two different parts: a) theory test with 30 multiple-choice questions, (60% of final grade); b) images exam with illustrations of anatomy atlases and natural anatomic pieces (30% of final grade).

Histology group: In this case the final grade of students enrolled in 2 different academic courses 2020–2021 (n=200) and 2018-2019 (n=211) were included in the study in order to compare a course in which Kahoot® was implemented (2020–2021) as a teaching/learning methodology with a “traditional” one (in terms of methodology, 2018-2019). Final exam is analogous to neuroanatomy with two different parts: a) theory test with 40 multiple-choice questions, (50% of final grade); b) images exam with micrographs of tissues (50% of final grade).

Our results after analyzing Kahoot® and final grade per student in Neuroanatomy, revealed a significant positive correlation with the theory test, image exam and the final grade ($r=0.334$ with $p<0.001$, $r=0.278$ with $p=0.002$ and $r=0.355$ with $p<0.001$, Figure 1). Regarding Histology, we compare the theory and image exam, as well as the final grade between two different academic years in terms of implementation of Kahoot®, all marks were significantly higher in the academic year using Kahoot® than the “traditional” year ($p<0.001$, $p<0.001$ and $p=0.014$ for U Mann Whitney test, respectively).

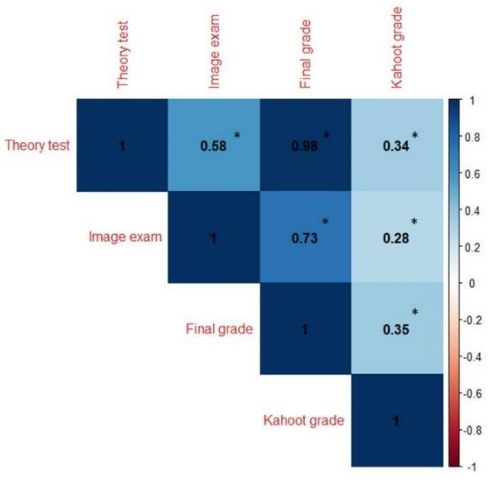


*Fig. 1. Correlation between Kahoot exam and different parts of final exam, *denotes p<0.05 calculated by Spearman method.*

Kahoot® has been proposed as a promise tool for facilitating formative assessment, however, so far, most studies have addressed it from a qualitatively perspective. To our knowledge, our results show Kahoot® as a powerful tool for evaluators to predict the acquired knowledge by the student. One of the remarkable aspects is that the effectiveness of this teaching technology as a formative assessment, can be transferable in their entirety, changing the content, to other subjects, as our results have demonstrated in Neuroanatomy and Histology. These results reveal Kahoot® as a valuable formative assessment tool in medical subjects.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Chatbots, a solution for degree subject FAQs

Del Valle E.[1,2], Martínez-Pinilla E.[1,2], Tolivia J.[1,2], García-Díaz M.[1,2] and Navarro A.[1,2]

[1] Department of Morphology and Cell Biology, University of Oviedo, Asturias, Spain; [2] Instituto de Neurociencia del Principado de Asturias, INEUROPA, Spain

Introduction: The use of intelligent chatbots as virtual assistant in customer support is being a revolution for any competitive business. This technological tool has also a big potential in the Education field. Bots can be very helpful in some tedious routine tasks associated to teaching. Along academic year, teachers receive numerous e-mails from our students with repetitive questions and doubts about the running of their subjects. Most of the answers can already be found in the syllabus or in the web page of the subject but anyway teachers spend considerable time replying to those frequent questions (FAQ). Therefore, we decided to build a chatbot based on artificial intelligence (AI) that would automatically respond to those questions.

Methods: We have created a chatbot based on AI using the IBM Watson platform to manage these FAQ. The use of this platform offers several advantages: it is located in IBM cloud, so you do not need to download any program; it is free (with fee-upgrading options) and it is not necessary to know any programming language to create the bot. First of all, you need to "train" your assistant in order to it to be able to answer the students' questions but being based on AI the bot is able to learn from experience and answer questions that we have not introduce to it.

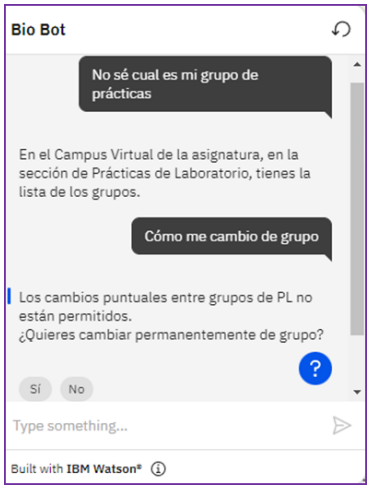


Fig. 1. An example of what the chatbot looks like.

Results: Students most frequent doubts are about which is their lab group, if they must repeat the lab practice in case, they are enrolled in the subject for the second time, if they can move from one lab group to another, ask for tutorials, etc. Until now, we have educated our bot in 21 different actions, introducing a minimum of 5 possible variations per action.

To "train" our bot, we have created a series of variables to determine the action contest to help to give a more precise answer to the student's question. The variables created have been: "call", "email", "exams", "and lab group", "theory group", "marks", "percentages", "professors". In case new needing would be detected, the suitable variables will be added. If the question is too complex or teacher's help is needed, the student will be forwarded to professor email.

Discussion & conclusions: The biggest advantage for teachers is the load work reduction, but also, they would be able to compile reports about the most frequent doubts and to detect information mistakes. For students, the biggest advantage is that they can solve they doubts immediately, regardless time, place, or the device they are using. Moreover, the chatbot is able to keep a conversation with several students at the same time. Maybe the weak point of the chatbot is the dehumanization of the process and the "cold" conversation of the bot, but a lot of improvement has been done regarding this aspect.

This chatbot will be part of a teaching innovation pilot study in the Cell Biology and Histology subject for the academic year 2022-2023

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

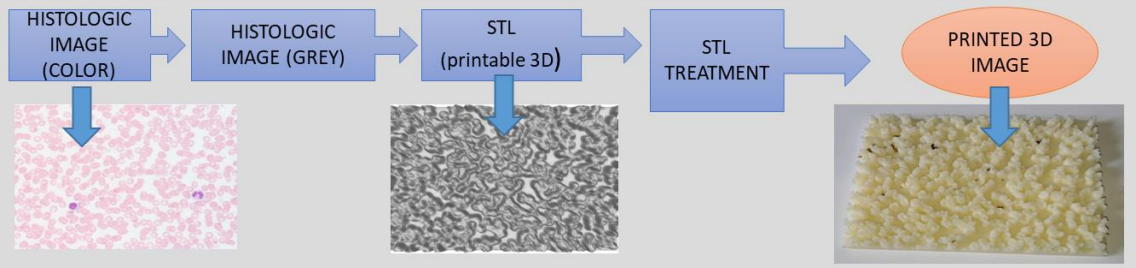
3D Histology: Beyond images

Bilbao E.[1,2*], Zaldibar B.[1,2], Díaz de Cerio O.[1,2] and Eguiraun H.[2,3]

1Department of Zoology and Animal Cell Biology, Leioa, University of the Basque Country, Spain; [2]Plentzia Marine Station (PiE), Plentzia, University of the Basque Country, Spain; [3]Department of Graphic Design and Engineering Projects, Bilbao, University of the Basque Country, Spain.

Histology studies the composition, structure and characteristics of living tissues, through the visualization and correct interpretation of images through the microscopic observation. In undergraduate teaching in Biosciences, visualization of histological images allows the integration of basic concepts such as cell, tissue and organ. Although no one questions the ability of images to convey large amounts of information quickly and concisely, students with vision problems, depending on the alteration type they present, may experience problems when interpreting histological images. In the extreme cases, these problems will not be corrected even with the use of digital scanners and/or by viewing through screens. For this reason, in the present work, a methodology that allows generation of tactile objects derived from histological images that could be used in teaching with people who have certain types of visual disabilities is stablished. These objects are built in a 3D printer from histological images obtained in color that undergo a series of transformations to be converted into 3D printable objects. The compromise between SCALING and the MINIMUM SIZE necessary for the image to be readable by touch will result in touchable histological images that could be helpful for visually impaired students in practical teaching in Biosciences.

Figure: diagram of essential steps to get printed histological 3D images.



Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Digital dynamization of learning in the practices of Human Histology through Wooclap as an interactive teaching methodology

Pascual G.[1,2,3], Pérez-Köhler B.[1,2,3], Benito-Martínez S.[1,2,3] and González-Santander M.[1]

[1] Departamento de Medicina y Especialidades Médicas, Facultad de Medicina y Ciencias de la Salud, Universidad de Alcalá, Madrid, Spain; [2] Biomedical Networking Research Centre on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN, ISCIII), Madrid, Spain; [3] Ramón y Cajal Health Research Institute (IRYCIS), Spain.

Introduction: The European Commission establishes, according to its policy, a tool based on the European Framework for the Digital Competence of Educators (DigCompEdu) [1] that insists on the need for a professional commitment of the teacher, through the insertion of digital resources that improve the acquisition of knowledge. This process includes the "alive and active" participation of students as a primary and essential agent, and not as a simple passive recipient. This way of being aware of and participating in their own learning, of constructing and integrating information, shapes the student's way of thinking and acting, which will imprint a way of "being" linked at all levels and states of life in lifelong learning [2]. It is teacher´s responsibility to provide methodological tools inducing a change in the "traditional" way of teaching, incorporating digitization media in real time and updating the teaching of "basic" subjects as Human Histology. This subject has considerable specific terminology and requires significant spatial integration, that is achieved in practical sessions through microscopic visualization in different sections and planes of space. It is the basis of knowledge for Organography (2nd year) and necessary to take the Pathological Anatomy (3rd year). Therefore, facilitating a good learned and integrated base allows the student to advance along a safe path in the correct acquisition of knowledge, participating in their own learning. Our strategy is to introduce Wooclap, as a new digital tool to interact with students and improve the quality of teaching, increasing motivation in the practical part of Human Histology.

Material and methods: Wooclap is a quick-to-use tool to interact in real time with our audience, improving the participation of students. It is highly versatile and can be easily applied to face-to-face, online, synchronous or asynchronous lessons. Involves the use of mobile phones, making it a very attractive tool for our students, where the mobile will become our ally. Students access the tool using an alphabetic code via web (www.wooclap.com) or by scanning a QR code. The tool has several functionalities allowing interaction by asking multiple-choice questions, polls, open questions, word clouds, numerical value questions, associations, image identification, etc., which teachers can use according to their needs. During the practical session, teacher provides the students with histological samples and guides their observations. In this pilot experience we have included four groups of approximately 20-25 students. After microscopic observation, they were anonymously subjected to different types of image-based Wooclap questions (*multiple-choice*, *tag in the image* and *locate in the image*), and a final question (poll type) to express their satisfaction and usefulness to check the acquired knowledge in the practical session. This experience is part of an active teaching innovation project granted by UAH (EV1309-2021).

Results: Wooclap shows automatic verification of the correct answers, which allowed student to check the achievement of the considered competences, the development of the observation capacity throughout the practical session, as well as decision making, what gives the student self-confidence. The type of questions showing the greatest difficulty for the student were those of *tagging in the image* with 70.6% of correct answers. The *multiple-choice* image identification questions yielded 85.1% correct answers, similar to the *locate in the image* questions that showed 85.6% correct answers. It must be considered that the student carries out the experience after a three-hour microscopy practise, without the possibility of consulting any information during the Wooclap pilot test. The students (97.8%) showed satisfaction with the new methodological strategy to stimulate practical sessions and to check the acquired knowledge in real time.

Conclusions: Wooclap is an optimal digital tool, that favors the acquisition of the practical competences of the subject and promotes a more active participation of the student.

[1] https://sede.educacion.gob.es/publiventa/marco-europeo-para-la-competencia-digital-de-los-educadores - digcompedu/competencia-digital/24685.
[2] Higher Education at the Crossroads of Disruption: the University of the 21st Century, A. Kaplan, Emerald, 2021.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Augmented Reality application as support for Cell Biology and Histology practices in Health Science subjects

Seco-Rovira V., Izquierdo-Rico M.J., Pastor L.M., Beltrán-Frutos E., Martínez-Alonso E., Jiménez-Movilla M., Ballesta J., Avilés M., Martínez-Menárguez J.A., Madrid J.F. and Ferrer C.

Department of Cell Biology and Histology. School of Medicine, IMIB-Arrixaca. Regional Campus of International Excellence. Campus Mare Nostrum. University of Murcia. (30120).

Introduction: The practices imparted by the Department of Cell Biology and Histology in Degrees of Health Sciences have the aim of getting students to relate the knowledge acquired in theoretical classes with the reality of the different cells, tissues and organs studied. To do this, students have access to classroom explanations and the Digital SlideBox tool to support them outside the practices. Nowadays, mobile devices give us access to a huge amount of information and there are numerous ways of learning about a wide variety of subjects, which is called Mobile Learning or M-Learning.

Objectives: the aim of this project is the development of an Augmented Reality (AR) environment, using the Zappar® application. This app allows students of the Bachelor's Degrees in Nursing and Dentistry to access the explanation of the objectives of each histological preparation at any time by scanning a Zapcode (application code). This technology will improve students' autonomous learning of the objectives of each practice and using mobile devices (M-Learning)

Methodology: Several days before the practice session, students were provided with an index of the slides to be studied in the practical session. Of the total number of slides, half were provided with a Zapcode (Fig.1) through which they could access the explanation of these slides with histological images and explanatory texts for each objective (new methodology). The remaining histological slides were explained in the practice session itself (classic methodology).

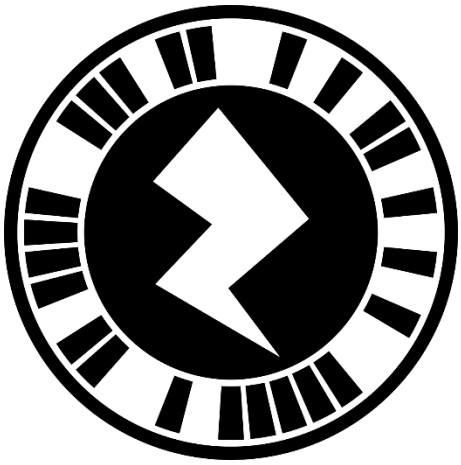


Fig.1. Example of Zapcode generated by the Zappar® app. With the app downloaded, you can scan it and access the corresponding preparation.

Results: To find out the degree of satisfaction after using one or other learning methodology (classic or new), students were given a survey at the end of the course consisting of 7 questions in which they were also asked about the usefulness of M-Learning in the subject and of Zappar as a tool for improving the study of the slides. Thus, the general degree of satisfaction with the Zappar application was more than 90% as good or very good, while when asked if this application made the teacher's explanation unnecessary, more than 85% did not agree or disagreed with it at all or not at all.

Conclusions: The students found the Zappar® app very useful as a way of achieving the objectives of the practices. They would include the Zapcode in all the slides of each practice. Moreover, the information is accessible from the mobile terminal making the study of the slides easier and more interesting.

SESSION

COMPARED HISTOLOGY AND ANIMAL HISTOLOGY

HISTOLOGÍA COMPARADA E HISTOLOGÍA ANIMAL

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Histopathologic changes in electrical conduction in sinoatrial and atrioventricular nodes following myocardial infarction in dogs and horses. Histological, morphometric and immunohistochemical study

Gómez-Torres F.A.[1], Ballesteros-Acuña L.E.[1] and Ruíz-Saurí A.[2]

[1] Department of Basic Sciences. Medicine School. Universidad Industrial de Santander. Bucaramanga, Colombia; [2] Department of Pathology, Faculty of Medicine, Universitat de Valencia, Valencia, Spain

Background: The conditions of the cardiac nodes are multiple and can generate different degrees of arrhythmias. The purpose of this work was to describe histologically and morphometrically the lesions that appeared in the cardiac nodes during the presentation of acute myocardial infarction and to compare them with normal tissues in dogs and horses.
Methods: In this study, we describe the histology and morphometry of the sinoatrial and atrioventricular nodes of 5 dog hearts and 5 elderly horse hearts that suffered sudden cardiac death. A computerized morphometric study was carried out, where we determined the number of cells that make up the nodes as well as different parameters related to their shape and size and the relationship with degenerative changes due to their cardiac condition. We performed a histological study with specific staining with hematoxylin-eosin, Masson's trichrome, and immunohistochemistry with antibodies against desmin to confirm cell identification.
Results: The sinoatrial node presented an ovoid shape and the atrioventricular node presented a pyramidal shape in both species. An increase in the percentage of collagen fibers was observed within the sinoatrial node of infarcted dogs (47%) compared to normal nodes (34.2%); Likewise, the sinoatrial node of infarcted horses showed a higher percentage of collagen fibers (50%) compared to normal animals (43.3%), which leads to a decrease in the percentage of cells. In the atrioventricular node, a decrease in the percentage of cells was observed in infarcted dogs (24%) compared to normal dogs (26.3%) and the same was found in infarcted horses (16%) compared to the percentage of cells in the interior of the node in normal horses (51.6%). In P cells from the sinoatrial node, we found that the area ($p=0.09$), maximum diameter (<0.001), and mean diameter (0.003) were larger in dogs than in horses. Analyzing the cells of the atrioventricular node, we observed that the area and diameters (maximum, minimum, and mean) of P cells, T cells, and cardiomyocytes were significantly greater in horses than in dogs ($p<0.001$ for all values). A decrease in the diameter of P and T cells was observed in the heart nodes of infarcted dogs compared to normal dogs. In infarcted horses, a decrease in the diameter of these same cells in the sinoatrial node and a hypertrophy in the atrioventricular node were observed compared to normal horses. When using the desmin stain to improve the identification of the nodal cells, we observed that it was only useful in the identification of the cells of the horse atrioventricular node when comparing them with the surrounding cardiomyocytes.
Conclusions: In general, sinoatrial node cells and surrounding cardiomyocytes in dogs and horses and atrioventricular node cells in dogs suffering sudden cardiac death were reduced in size relative to that reported in normal hearts. Atrioventricular node cell hypertrophy was observed in horses with sudden cardiac death.

Keywords: sinoatrial node, atrioventricular node, myocardial infarction, dog, horse

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Histological and morphometric changes in cardiac conduction fibers in myocardial infarction as compared to normal cardiac tissues in horses and dogs

Gómez-Torres F.A.[1], Ballesteros-Acuña L.E.[1] and Ruíz-Saurí A.[2]

[1] Department of Basic Sciences. Medicine School. Universidad Industrial de Santander. Bucaramanga, Colombia; [2] Department of Pathology, Faculty of Medicine, Universitat de Valencia, Valencia, Spain

Background: Diseases of the cardiac conduction system in myocardial infarction often induce arrhythmias that can aggravate the patient's condition. The purpose of this work was to describe by histology and morphometry the changes in cardiac conduction fibers and their cells, after an acute myocardial infarction and to compare them with normal cardiac tissues in horses and dogs.
Methods: In this study, we describe the histology and morphometry of cardiac conduction fibers and their cells in 5 dog hearts and 5 elderly horse hearts that suffered sudden cardiac death. A computerized morphometric study was carried out, where we determined the density and thickness of the fibers, as well as the area and diameters of the cardiac conduction cells, comparing them with the data described in hearts without cardiac involvement. Histological study was performed with specific staining with hematoxylin-eosin and Masson's trichrome.
Results: When studying the cardiac conduction cells in horses, we found that in the cases of hearts that did not suffer myocardial infarction, the area and diameter of these cells were greater than in the cases where the hearts presented myocardial infarction ($p<0.001$ for all values) (Table 1). In dogs, it was observed that the area, the maximum diameter and the mean diameter were significantly greater in the hearts that suffered sudden death due to myocardial infarction ($p<0.001$ for all values), the same happened with the minimum diameter ($p=0.002$) and roundness (0.001), observing a hypertrophy in these cells compared to normal (Table 1).

	Area (μm²)		Max. Diam (μm)		Min. Diam (μm)		Mean Diam (μm)		Roundness	
	Horse	Dog	Horse	Dog	Horse	Dog	Horse	Dog	Horse	Dog
Myocardial infarction	1,882.94	463.96	55.74	26.83	37.08	19.46	45	22.90	1.21	1.13
Not infarcted	3,560.88	363.46	77.40	23.92	53.74	17.65	63.98	20.59	1.20	1.11

The density (25.96%) ($p<0.001$) and the thickness (57.35 μm) ($p<0.001$) of the cardiac conduction fibers in horses that did not suffer myocardial infarction were significantly greater than in the cases that presented sudden death and myocardial infarction. cardiac (7.28% and 31.79 μm respectively). In dogs, the density (17.39%) ($p<0.001$) of cardiac conduction fibers was greater in hearts that did not present myocardial infarction, compared to infarcted cases (8.92%) and the thickness of these fibers was greater in dogs infarcted cases (31.86 μm) ($p<0.001$) than in normal cases (20.14 μm), indicating hypertrophy of these fibers. The area of infarction was observed mainly in the posterior region in horses with the presence of coagulative necrosis of the muscle fibers and a wide extension of scar tissue that replaces the cardiac muscle tissue. In dogs, a distribution of the infarct zone was observed in all regions of the heart, presenting a slight loss of continuity of cardiac fibers, a slight presence of scar tissue and the presence of some inflammatory cells.
Conclusions: The decrease in the percentage of cardiac conduction fibers observed in horses and dogs after acute myocardial infarction could be explained by the progressive decrease in myocardial perfusion before the occurrence of the acute coronary event.

Keywords: myocardial infarction, conduction cardiac fibers, dog, horse

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Morphological study of the caprine aortic valve system: An anatomical-histological model for the study of valve pathology

García-Palomeque J.C.[1], Larran J.[1], Guzmán J.L.[1,2], Molero-Chamizo A.[3] and Salido M.[1]

[1] Histology Department, School of Medicine, Cádiz University, Spain; [2] Agroforestry Sciences Department, Huelva University, Spain; [3] Psychobiology Area, University of Huelva, Spain

Introduction: Knowledge of anatomy and morphology plays an important role in the study of tissue, cellular, and molecular changes that occur in various valvular pathologies such as endocarditis, heart failure, and valvular stenosis. The study of the aortic valve, as an experimental model, requires a detailed histological description that is essential for its possible translation. The most relevant experimental models of the aortic valve are rodent models, but there are no standard models in other animal species [1]. Thus, we describe here a goat model for the study of the aortic valve. Among the advantages of this model is that the size and structure of the caprine valve are similar to those of the human being, and histologically it shows the morphology of epithelial trilayers described in humans.
Methods: Through this model we analyze possible changes in the aortic valve after an experimental intervention that has been shown to be effective in other experimental models. In particular, we analyzed the possible induction of degenerative aortic stenosis through changes in the animals' diet. A histological study of the cell population and connective structures of the aortic valve and caprine cardiac tissue, both from newborns and adult goats, was carried out by carving the aortic valve and the subvalvular apparatus. In the histological and microscopy procedure, the following staining procedures were performed: hematoxylin-eosin, Masson's trichrome stain, and Orcein stain. The immunohistochemical procedure was used to mark different cell subtypes, and transmission microscopy was also used to analyze the different structures of the connective tissue at the subcellular level.
Results: The results indicate that the goat model used is useful to show experimentally induced histopathological changes in the aortic valve, similar to the changes described in rodent models.
Discussion & conclusions: Unlike the rodent model, caprine valve histology shows the same morphological organization of epithelial trilayers as that of humans.

[1] Fernández B, et al. Vet Pathol. 2020;57(2):321-331

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Some morphological patterns on rabbit cecal patch

Pérez F.A.[1], D'Ottavio A.E.[1;2] and Roma S.M.[1;2]

[1] Department of Histology and Embryology, Faculty of Medical Sciences, National University of Rosario, Argentina; [2] Research Council National University of Rosario, Argentina

Introduction: Several morphological patterns regarding rabbit appendix (A) and Peyer's patches (PP) were previously analyzed in our laboratory. Completing now those findings, the cecal patch - other main immune-inductive site of its digestive tube - was studied. **Aim of this communication:** The present report summarizes some related preliminary results in that organ. **Material and method:** Cecal patch samples were obtained from ten adult New Zealand rabbits. Firstly, as no data were registered in relation with the origin of its membranous cells (M cells), located in the follicle associated epithelium (FAE) and specialized in the uptake of luminal particles, immunostaining with Vimentin (universal and reliable marker for M cells) and Ki-67 (proliferation marker) were used. Since the cryptal position establishes the stage of cell differentiation, the crypts were examined through laser confocal scanning microscopy for determining the position of the cells showing cytoplasmic filaments (Vimentin+) and that of the cells with proliferating nuclei (Ki-67+). Both were counted in increasing, ascending and consecutive order from the cryptal basal zone (position 0) to its apical one. Following this direction, the position of the first and the last Ki-67 (+) nucleus and the first Vimentin (+) cell were considered for cell proliferation and for M cells origin, respectively, as well as the position of the cells where both phenomena overlapped. Furthermore, the two existing phenotypes of M cells - one, mature with lymphocytes in its membranous pockets and other, immature, lacking them - were also identified with Vimentin. Finally, the location of specific glycoside residues in FAE, implicated in the surface adhesion of macromolecules, was put into evidence employing two lectins: Ulex Europaeus Agglutinin I (UEA-I) for L-fucose and Dolichos Biflorus Agglutinin (DBA) for N-acetylgalactosamine. When necessary, the Kruskal-Wallis test was used for statistical analysis. **Results:** Cecal patch crypts exhibited filamentous vimentin positivity (ns), similar to A and PP, indicating an early differentiation with already committed cells to the M cell line in the mid-crypt. Conversely to those organs, proliferation in the cecal patch was detected deeply and extended higher towards the mouth of the crypt (p=0,0031). Likewise, immature M cells were more notorious in cecal patch than in the two abovementioned organs. Regarding lectins, DBA showed a diffuse positive stain including FAE, the lymph follicle and its connection zone whilst UEA-I evidenced no differences among cecal patch, A and PP. **Conclusion:** (1) M cells in the cecal patch are also generated from undifferentiated cryptal stem cells and not from mature enterocytes, as frequently supported; (2) higher crypt proliferation may be stimulated by the cecal microenvironment, rich in luminal bacteria, and possibly reflected in the increase of FAE immature M-cells; (3) the strong presence of L-fucose may point out a local adaptation to a specific luminal milieu as cecum is with its noticeable microbiota.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

A comparative study of the retinal specializations in two bird species: *Falco tinnunculus* (Linnaeus, 1758) and *Burhinus oedicnemus* (Linnaeus, 1758)

Navarro-Sempere A., Segovia Y., García-Fernández E., Jiménez-Díaz D. and García M.

Department of Biotechnology, University of Alicante

Vision is the primary and most highly developed sense in birds. In response to the diversity of habitats shared by birds, their visual system has evolved in a highly selective manner, developing a series of structural and functional adaptations that have allowed them to achieve a visual acuity far superior to that of other vertebrates. These specialisations include the presence of regions of maximum visual acuity, such as areas and fovea, as well as the presence of the *pecten oculi*. The information received about the habitat will define the characteristics of the retina and the different types, numbers and positions of retinal specialisations, which coincide with the areas of highest visual acuity and which allow us to obtain greater information about the visual space.

The present work is focused on describing the morphological retina structure of a diurnal species, the common kestrel (*Falco tinnunculus*) and a nocturnal one, the stone-curlew (*Burhinus oedicnemus*), as well as the histological adaptations they present in relation to lifestyle. Furthermore, this study provides valuable information about the vision of predatory birds.

In order to evaluate this, four ocular globes from two adults' specimens of each species were obtained from Santa Faz Wildlife Recovery Centre (Alicante). Following the enucleation, eyes were dissected and their anterior portion was cut away. Right retinas were processed for transmission electron microscopy, while left retinas and *pecten oculi* embedded in paraffin for light microscopy. Each retina was divided into the central and peripheral zones. The orientation of each zone was made following the *pecten oculi* position. Foveae were located and processed. Measurements of the thickness of the entire retina and of each retinal layer were determined every 20 µm across of each sample and averaged.

In both species, the thickness of the central retina was higher than the peripheral (290,68 ± 0,89 µm vs 140,38 ± 1,02 µm in the common kestrel and 241,26 ± 1,82 µm vs 137,30 ± 1,66 µm in the stone-curlew). Also, the most of retina layers showed differences in the thickness between central and peripheral region in the two studied species. As occurs in other predator birds, in *F. tinnunculus* and *B.oedicnemus* the increased thickness in the central region is mostly due to a higher density of cells in the inner nuclear layer of the retina and higher complexity of neurons arborization in the inner plexiform layer. Regarding retinal specializations, the kestrel is a bifoveated animal, it shows a deep central fovea and a shallower, peripheral one as previously described in other diurnal birds of prey. The stone-curlew presents only one central fovea, shallower than the fovea from the *F.tinnunculus*, as in other nocturnal raptors. The central fovea is linked to monocular lateral vision. This type of focus allows the bird to detect and focus on prey from a long distance and is most profound in birds capturing fast-moving prey or food [1,2,3]. The temporal fovea, related to binocular vision, is important for prey fixation at the time of capture. On the other hand, the pecten oculi of diurnal and nocturnal birds of prey is folded type, varying mainly in number of folds between the two. Diurnal birds have a larger pecten oculi with a greater number of folds than nocturnal birds. In this study, the *pecten oculi* of the common kestrel showed a folded morphology with 16 folds, while that of the stone-curlew had 8 folds.

In conclusion, both analyzed species presents retinal specializations related to the predatory lifestyle such as the variation of the thickness along the whole retina or the presence of, at least, one fovea, however the two species showed certain adaptations to the diurnal/nocturnal habits: type of predominant photoreceptor cells or the fold number in the *pecten oculi*.

This research was supported by the University of Alicante VIGROB-186

[1] Reymond, L. (1985). Vis. Res. 25(10): 1477-1491.
[2] Reymond, L. (1987). Vis. Res. 27 (10): 1859–1874.
[3] Jones, M., Pierce, K. and Ward, D. (2007). J. Exot. Pet Med. 16(2): 69-87.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Stimulation of NETs and myeloperoxidase granules release of gilthead seabream head kidney leucocytes

Albaladejo-Riad, N.[1], Duquet, A.[2], Collado-González, M.[1], Cuesta, A.[1] and Esteban, M.A.[1]

[1] Immunobiology for aquaculture group. Department of Cell Biology and Histology. Faculty of Biology, University of Murcia, 30100 Murcia, Spain. Email: nora.albaladejo@um.es; [2] *Ecole Supérieure de Biologie-Biochimie-Biotechnologies* (ESTBB). Université Catholique de Lyon. Lyon, France.

Introduction: The interest of the immune responses developed by granulocytes is increasing at present. The gilthead seabream (*Sparus aurata*) head kidney has been used as a neutrophil supply for studying the degranulation and formation of neutrophils extracellular traps (NETs) by using different stimuli.
Methods: Leucocytes were isolated from the kidney of *S. aurata* (n=6) and maintained in culture for check the efficiency of phorbol myristate acetate (PMA), *Escherichia coli* lipopolysaccharides (LPS), β-glucan (BG), calcium ionophore (CaI) and polyinosinic:polycytidylic acid (Poly I: C) to stimulate the release of myeloperoxidase (MPO) from the granules and NET's formation, adapting an absorbance and a fluorescence (SYTOX Green-labelled extracellular DNA) assays, respectively. Neutrophils were stimulated with different concentrations of the named substances and several times (ranging from 10 to 180 minutes). Subsequently, and according to the best results obtained in the concentration and time sweep, the leucocytes were stimulated with the corresponding reagent at a given concentration (0.5 µg/mL PMA; 10 µg/mL LPS; 400 µg/mL BG; 5 µg/mL CaI; 5 µg/mL CaI and 200 µg/mL Poly I;C) for 60 min, and the stimulated cells were studied by confocal microscopy (immunohistochemistry), brightfield microscopy (Giemsa) and scanning electron microscopy (FSEM). To perform the immunohistochemical staining, we labelled the DNA with DAPI and the cell membrane with a specific membrane antibody for gilthead seabream, the FITC-conjugated D2 antibody. To support the results obtained above, photographs of the NETs were taken for confocal microscopy with a Leica STELLARIS 8 confocal microscope, for brightfield microscopy a Leica upright microscope (DM6000B) and for FESEM we used Apreo S. Image analyses were conducted with Fiji software. Statistical analyses were carried out with a two-way ANOVA ($p<0.05$; Tuckey) using GraphPad 9.
Results: The results obtained show that after 1 hour of incubation, the most effective stimulant for NET formation and myeloperoxidase granules in gilthead seabream head kidney leucocytes is PMA ($p< 0,05$) at a concentration of 0.5 µg/mL. Although the fluorescence assays supported the highest DNA release with Poly I:C, microscopy images have shown that this was due to a complete release of the nucleus out of the cells.
Discussion & conclusions: These results show the standardization of an *in vitro* assay to measure NETs release in seabream leucocytes, selecting the best stimuli with the support of microscopy, as well as the standardization of an immunohistochemical protocol to visualize the NETs formation. In addition, it is known that gilthead seabream leucocytes lack TLR4, so they should not have been stimulated with LPS at any time. These results have demonstrated that there must be some indirect route by which LPS stimulates the activation of gilthead seabream acidophilic granulocytes. New studies are needed to demonstrate this hypothesis.

NAR has a PhD grant (FPU18/02544). This research has been financed by the *Proyecto* PID2020-113637RB-C21 *de investigación financiado por* MCIN/ AEI /10.13039/501100011033.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

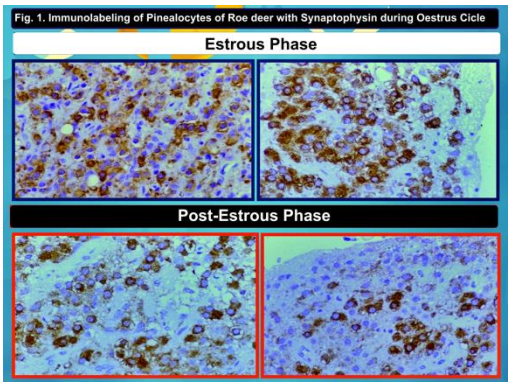
POSTER PRESENTATION

The oestrous cycle as a modulator of the histophysiological modifications of the pineal gland of the Roe deer

Redondo-García E.[1], Sánchez-Porro A.[2] and Masot-Gómez-Landero A.J.[1]

[1] Animal Medicine Department, Histology, University of Extremadura, Cáceres, Spain; [2]Extremadura Health Service, Don Benito Regional Hospital, Badajoz, Spain

Introduction: In the populations of our study area, the rut of the roe deer takes place in the second half of July and the births occur between end of april and end of may. Days after copulation, the female initiates a delayed implantation process, very similar to a suspension of the pregnancy itself. If this phenomenon did not occur, the neonatal roe deer would be born in the middle of winter, with extreme cold and a reduction of nutrients, which would lead to possible death. The resumption of gestation is december-january, so each female will give birth in the months of april-may [1]. The roe deer as a photoperiodic animal needs to adapt its reproductive functions to the daily and seasonal variations of external factors (light, food, etc.). These adaptive processes require: recognition of these variations; translate them into hormonal messages; and translate them to develop adaptive responses. In the roe deer, these adaptive processes involve three main structures: the retina, the hypothalamus, and the pineal gland. The pineal is responsible for receiving information about the start of the dark phase. All this is due to the secretion of the melatonine hormone (MT). When night begins, norepinephrine released by the superior cervical ganglion stimulates pinealocytes to MT [2]
Methods: 1º.- morphological parameters of the pineal gland of roe deer were quantified, in estrous phase and in post-estrous periods, by immunodetection and histomorphometric quantification of pinealocyte and interstitial cell markers; 2º.- the modulating effect of MT on the histophysiology of the pineal was determined, in oestrous cycle; and 3º.- the modifications in MT contents, in plasma (by RIA) and in the pineal parenchyma (by HPLC) were evaluated, in estrous and post-estrous conditions of roe deer killed at different times.
Results: 1. All the glands of the roe deer killed during the night, had significantly greater weight, length and volume, than that of the animals sacrificed during the day (Tukey test: $P < 0.001$; $P < 0.001$; and $P < 0.003$,). In addition, the weight of the pineal glands of the killed animals in estrous phase, both in the period of light and in the dark, showed significant increases, compared to that of animals killed in the post-estrous ($P < 0.002$). 2. The number of pinealocytes and interstitial cells in estrous was significantly higher than in post-estrous ($P < 0.003$; and $P < 0.001$). The number of SYNAP+ and GFAP+ cells was significantly greater in medulla than in cortex, for both groups ($P < 0.002$; and $P < 0.001$). In periods of light/darkness, in roe deer killed in post-estrous, there were no significant differences in terms of the number of pinealocytes and interstitials cells; quite the opposite that happened in roe deer killed in estrous ($P < 0.0002$; and $P < 0.0001$). 3. The concentrations in MT of the pineal, as well as the plasma levels of MT, increased significantly during the night, both in estrous and post-estrous periods ($P<0.0001$, $P<0.0002$; and $P<0.0002$, $P<0.0003$, for HPLC and RIA, respectively). MT concentrations in the pineal glands of roe deer in estrous, both day and night were significantly different to those of the post-estrous ($P< 0.0003$; $P< 0.0002$).



Discussion & conclusions: 1.- The pineal gland of the roe deer, during the estrous period, was shown as a very active structure, both from the morphological prism and from the functional. 2.- MT, during the roe deer's oestrous cycle, modulated the histophysiological response of the pineal gland, regulating glandular cytology (pinealocytes and interstitial cells), and moderating the secretions of this hormone by pinealocytes.

Supported by Junta de Extremadura-FEDER (GR 21060 and IB 20008).

[1] Stone DB, et al. PLoS One. 2017; 12(6): e0178477
[2] Lépinay J, et al. PLoS One. 2021; 16(7): e0255249

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Differential ciliogenesis in the different epithelial cell components of human and rat thyroid glands

Vázquez-Román V.[1], Pérez-Fernández B.[1], Fernández-Santos J.M.[1] and Martín-Lacave I.[1]

[1] Department of Normal and Pathological Cytology and Histology, School of Medicine, University of Seville, Spain.

Background: We recently described that primary cilia (PC) are present in the human thyroids, where they extended from the follicular cell apex into the lumen [1, 2]. PC, taking advantage of this localization, may sense the colloid environment, and this sensory activity, coupled to intracellular signaling pathways, contributes to the complex mechanism of thyroid hormonogenesis, being PC role investigated in thyroid [3]. Nevertheless, thyroid gland possesses two other less represented epithelial-cell components, specifically, C cells (CC) and ultimobranchial (UB) remnants, whose ciliogenesis is completely unknown. Therefore, the first objective of the present work was to solve that gap in the knowledge of the thyroid gland. Secondly, we aimed to extend the same analysis to the rat thyroid gland, to validate it or not as an experimental model for studying the behavior of PC in different functional thyroid states.
Methods: Normal thyroids from humans (n=3) and Wistar rats (n=6) were either paraffin-embedded and serially-sectioned, or processed for transmission electron microscopy (TEM). Paraffin sections were immunostained for calcitonin (CT) or 34βE12-cytokeratin (CK) to identify CC or UB remnants, respectively. Consecutive sections were immunostained by double immunofluorescence (IF) to demonstrate PC -using acetylated α-tubulin for axoneme or γ-tubulin for basal body - together with either thyroglobulin (TG), CT or CK, followed by Cy3 or Cy2-labelled IgG antibodies.
Results and Discussion: Human thyrocytes exhibit long PC at the apical pole that plunge into the colloid. In contrast, in rat thyroids, PC are very difficult to distinguish at the apex of follicular cells. Therefore, we analyzed PC using double IF for both axoneme and basal body, where PC become recognizable but rather short and scarce. These data were confirmed at TEM level. This kind of PC has been referred as “procilia” by some authors [4]. In relation to CC, it occurs just the opposite than before: PC are easily observed in the numerous CC of rat thyroid gland, in contrast with human CC, where PC are rarely seen. This is the first time that PC are described in CT-producing cells. Finally, in relation to UB remnants, these structures are rather difficult to recognize by H-E, either in humans (“solid cell nest”/SCN) or in rat (“UB follicles”/UBF). Therefore, we first identified them using a specific marker –anti-334βE12-CK and IHC [5]. Then, in contiguous sections, a double IF was carried out. PC appeared in both SCN and UBF, although very sporadic and mostly in peripheral UB cells. Conclusions: The pattern of ciliogenesis in both human and rat thyroid glands differs considerably, therefore, in our opinion, the Wistar rat is not a valid experimental model to analyze the behavior of PC after inducing different functional or pathologic thyroid states.

[1] Utrilla J.C., et al. (2015). Comparative study of the primary cilia in thyrocytes of adult mammals. J. Anat. 227/4, 550-50,
[2] Fernández-Santos J.M., et al. (2019). Primary Cilium in the Human Thyrocyte: Changes in Frequency and Length in Relation to the Functional Pathology of the Thyroid Gland. Thyroid 29/4, 595-606,
[3] Lee J., et al. (2021). Primary Cilia Mediate TSH-Regulated Thyroglobulin Endocytic Pathways. Frontiers in Endocrinology, 12: Article 700083.
[4] Alieva I.B. and Vorobjev I.A. (2004). Vertebrate primary cilia: a sensory part of centrosomal complex in tissue cells, but a “sleeping beauty” in cultured cells? Cell Biol. Int. 28,139–150.
[5] Vázquez-Román V., et al. (2013). Postnatal fate of the ultimobranchial remnants in the rat thyroid gland. J Morphol 274/7:725-32.

Supported by CTS- 439/2017 (Diffuse Neuroendocrine System).

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Pupillary reflex and development of eye movements in lampreys

Jiménez-López C.[1], Barandela M.[1], Núñez-González C.[1], Megías M.[2], Pombal M.A.[2] and Pérez-Fernández J.[1]

[1] CINBIO, Neurocircuits group, Universidade de Vigo, 36310 Vigo, Spain. [2] Departamento de Bioloxía Funcional e Ciencias da Saúde, Facultade de Bioloxía-IBIV, Grupo Neurolam, Universidade de Vigo, 36310 Vigo, Spain.

To use the visual system for advanced behaviors, different types of eye movements have evolved through new motor and perceptual needs. The first movements appeared to immobilize the image on the retina: the vestibulo-ocular reflex (VOR), which ensures eyes stabilization during head movement, and the optokinetic reflex, which keeps the visual field static on the retina when it is shifted. On top, goal-oriented eye movements redirect gaze to specific objectives. Additionally, the pupillary reflex allows to adapt the amount of light that enters the eye, contributing to appropriate image formation. However, the origin and evolution of the circuits involved in these processes are unclear.

Lampreys, belonging to the oldest group of living vertebrates, have a relatively simple nervous system but with many basic features that have been conserved through vertebrate evolution. The visual system is well developed, and lampreys have image-forming eyes and the same basic neuronal systems controlling visuomotor behaviors, as well as the basic types of eye movements. This facilitates the dissection of these conserved neuronal circuits shedding light on the mechanisms present in all vertebrates.

The lamprey visual system develops stepwise during its larval period, and this development is thought to reflect its evolution. Although larvae have immature, skin-covered eyes, the neural circuits underlying the VOR appear in this period, although it is not known whether functional eye movements are present. On the other hand, the optic tectum, which is a major visual center that controls gaze direction, is immature in larvae, although retinal inputs are established in late larval stages. Regarding the pupillary reflex, it is mediated by the Edinger–Westphal nucleus via the ciliary ganglion in mammals. Besides, a second slower mechanism may also be present, mediated by melanopsin from the fibers of the sphincter pupillae muscle, whose conformation changes with light, causing pupil reduction. The existence of the pupillary reflex has recently been reported in lampreys, but its mechanisms have not yet been determined.

Our aim is to study when different types of eye movements appear and analyze their underlying neuronal mechanisms and unveil the mechanisms underlying the pupillary reflex, to shed light about the evolutionary origin of theses visual functions. Our results show that eye movements are already present in larvae in the form of VOR and that, at least the intrinsic mechanism of pupillary reflex occurs in lamprey. These results indicate that stabilizing eye movements appear earlier than goal-oriented ones, and that at least the intrinsic pupillary reflex was already present in early vertebrate evolution.

Acknowledgements: Projects I+D+i PID2020-113646GA-I00 funded by MCIN/AEI/ 10.13039/501100011033 and by “ERDF A way of making Europe”, the Ramón y Cajal grant RYC2018-024053-I funded by MCIN/AEI/ 10.13039/501100011033 and by “ESF Investing in your Future”, and Xunta de Galicia (ED431B 2021/04).

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Alteration of gilthead seabream (*Sparus aurata*) hepatocyte morphology induced in the early stage of *Vibrio harveyi* infection

Serna-Duque J.A., Espinosa C., Albaladejo-Riad N. and Esteban M.A.

Immunobiology for Aquaculture Group, Department of Cell Biology & Histology, Faculty of Biology, University of Murcia, Murcia, Spain.

Vibriosis is one of the most widespread bacterial diseases with the highest mortality rates in intensive marine fish farming. One of the best-known bacteria causing this disease is *Vibrio harveyi*, which is highly endemic in the microbiota of the seas and in farmed fish. Naturally, this bacterium does not pose a risk to animal health, however, under conditions of high fish density as is often used in aquaculture, it becomes a serious opportunistic pathogen. One of the species affected is gilthead seabream (*Sparus aurata*), a species of great importance in Mediterranean aquaculture. In this study, fish were intraperitoneally injected with *V. harveyi* and PBS-injected fish were used as controls, and liver samples were collected and fixed in formalin (10%) at 4h post-injection, to study the effect of bacterium in early-stage infection in the hepatocytes. Liver samples were embedded in paraplast and its blocks were sectioned at 5 µm. These sectioned samples were stained with haematoxylin-eosin and mounted in DPX. Images were acquired with a Leica DFC280 digital camera attached to light microscope (Leica 6000B) at 50 µm. Microscopic images of liver indicated relevant alterations in hepatocytes of Vibrio-injected seabreams. Hepatocytes showed vacuolated cytoplasm and nuclei were displaced to periphery. Likewise, disorganization of parenchyma was evident. Additionally, image analysis was performed by Fiji software to measure % of vacuolated area in the hepatocytes. These results indicate a significative increase of 47% in vacuolization (t-student, $p < 0.05$) of infected liver compared control liver, from 13% to 19 % of vacuolated area. Histologic study of liver stimulated with pathogenic bacteria indicates serious alteration of hepatocytes due to metabolic stress. The liver is known to be the main supplier of acute phase proteins and the experimental infection provoked by this pathogenic bacterium could have stimulated protein synthesis in gilthead seabream hepatocytes, which could be visualized as the increased vacuolization observed. This vacuolization could perhaps be due to the metabolic stress caused.

JASD has a PhD grant (FPU19/02192). Proyecto PID2020-113637RB-C21 de investigación financiado por MCIN/ AEI /10.13039/501100011033.

[1] B. Weber, C. Chen, D.L. Milton, Environ. Microbiol. 2010; Rep.2 133–139.
[2] B. Austin, Vet. Microbiol. 2010; 140, 310–317.
[3] E. Chaves-Pozo, P. Muñoz, A. López-Muñoz, P. Pelegrín, A. García Ayala, V. Mulero, J. Meseguer, Cell Tissue. 2005; Res. 320, 61–68.

SESSION

NERVOUS SYSTEM

SISTEMA NERVIOSO

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

HB-EGF participates in the ERBB signaling pathway inducing chemotaxic migration and invasive properties of neural stem cells

Romayor I.[1], Manero-Roig I.[1,2], Polo Y.[3], Luzuriaga J.[1], Basanta R.[1], Pastor-Alonso O.[4], Encinas J.M.[4], Eguizábal C.,[5,6] Unda F.[1], Ibarretxe G.[1] and Pineda J.R.[1,4]

[1] Cell Biology and Histology Department, University of the Basque Country (UPV/EHU), Leioa, Spain; [2] Université de Bordeaux IINS - UMR 5297, Bordeaux, France; [3] Polimerbio SL, Donostia-San Sebastian, Spain; [4] Achucarro Basque Center for Neuroscience Fundazioa, Leioa, Spain; [5] Cell Therapy, Stem Cells and Tissues Group, Biocruces Bizkaia Health Research Institute, Barakaldo, Spain; [6] Research Unit, Basque Centre for Blood Transfusion and Human Tissues, 48960 Galdakao, Spain.

Introduction: Heparin-binding epidermal growth factor-like growth factor (HB-EGF) is a heparin-binding member of the ErbB receptors [1] activating Her1 and Her4 [1,6,7]. HB-EGF is a potent mitogen and chemotactic factor for fibroblasts and smooth muscle cells [2,3,4]. Moreover, HB-EGF has been shown to stimulate stromal proliferation [8] and migration [9]. In central nervous system, HB-EGF has been found to be mitogenic for astrocytes [5] although its role in adult neural stem and progenitor cells (NSPCs) is completely unknown.
Methods: Intrahippocampal administration of kainic acid was driven by stereotaxic injection and HB-EGF content by ELISA was determined using DuoSet Immunoassay (R&D) as previously described [10,11]. Immunofluorescence against HB-EGF (1:200) was performed either in sections of 50 μm thickness of the dentate gyrus from Nestin-GFP animals *in vivo* or in NSPCs cultures previously seeded on 1:100 laminin coated coverslips. RT-qPCR was performed as described [12]. Effect of HB-EGF blockage over Her1 receptor was done with 2h pre-incubation of 2μM of Gefitinib. For Western blot analyses, samples were collected in RIPA buffer with protease and phosphatase inhibitor cocktail loading 20μg of protein into 10% Tris-Glycine gels. Phospho-specific antibodies against EGFR (Tyr1068, Tyr845), ERK (Thr202/Tyr204) and control antibodies against total EGFR, ERK were used. Transwell invasion assay and videomicroscopy were done as previously described [13].
Results: Using a mouse model of mesial temporal lobe epilepsy we detected increased immunolabeling for HB-EGF 3dpKA in the SGZ and the portion of the molecular layer closer to the GCL of Nestin-GFP mice. We analyzed HB-EGF levels by ELISA at different time points detecting a significant increase in the hippocampus at 12hpKA and 24hpKA, decreasing to control levels at 72hpKA. Primary cultures of dissected NSPCs expressed ErbB receptors by RT-qPCR, co-stained for HB-EGF and were able to release HB-EGF to the culture media. NSPCs cultured in presence of 50 ng/mL of HB-EGF did not altered drastically their migration velocity and the pausing time; however transwell invasion assay they showed a more invasive phenotype.
Discussion & conclusions: These results suggest that HB-EGF participates in the ERBB signaling pathway acquiring chemotaxic migratory and invasive properties of adult neural stem cells.

Supported by UPV/EHU (PPGA20/22) Basque Government Health Department (Grant 2021333012), MICINN/AEI/10.13039/50110001103 (PID2019-104766RB-C21 & MINECO RYC-2013-13450). B. Pardo-Rodriguez has a predoctoral funding for non-doctoral Research Staff from the Basque Government (Ref. 2021_1_0112). Polimerbio and Y. P. have a Bikaintek PhD grant (20-AF-W2-2018-00001). I.M. have a UPV/EHU mod-III grant. I.R have a Margarita Salas Grant (MARSA21/60).

[1] Raab G, Klagsbrun M. Biochim Biophys Acta, 1997, 1333: F179-99
[2] Blotnick S, Peoples GE, et al. Proc Natl Acad Sci USA, 1994, 91:2890-4
[3] Fukuda K, Inui Y, Kawata S, et al. Arterioscler Thromb Vasc Biol, 1995, 15:1680-7
[4] Dluz SM, Higashiyama S, Damm D, Abraham JA, Klagsbrun M. J Biol Chem 1993, 268:18330-4
[5] Jia M, Shi Z, Yan X, Xu L, Dong L, Li J, et al. J Neurosci Methods. 2018, 307:240-7.
[6] Graus-Porta D, Beerli RR, Daly JM, Hynes NE. EMBO J. 1997, 16:1647-55
[7] Iwamoto R, Mekada E. Cytokine Growth Factor Rev. 2000, 11:335-44
[8] Hisaka T, Yano H, Haramaki M, Utsunomiya I, Kojiro M. Int J Oncol. 1999, 14:453-60
[9] Faber-Elman A, Solomon A, Abraham JA, Marikovsky M, Schwartz M. J Clin Invest. 1996, 97(1):162-71
[10] Sierra A, et al. Cell Stem Cell. 2015, 16(5):488-503.
[11] Canals JM, Pineda JR et al. J Neurosci. 2004, 24(35):7727-39
[12] Pineda JR, Daynac M et al. EMBO Mol Med. 2013, 5(4):548-62.
[13] Polo Y, Luzuriaga L, et al. Nanomedicine. 2021, 31:102314

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

The hypothermia mimetic synthetic molecule zr17-2 prevents retinal damage caused by perinatal asphyxia in the rat

Rey-Funes M.[1], Fernández J.C.[1], Peláez R.[2], Soliño M.[1], Contartese D.S.[1], Ciranna N.S.[1], Nakamura R.[1], Dorfman V.B.[3], Zapico J.M.[4], Ramos A.[4], de Pascual-Teresa B.[4], López-Costa J.J.[1], Larrayoz I.M.[2], Martínez A.[2,¶] and Loidl C.F.[1,¶]

[1]Instituto de Biología Celular y Neurociencia "Prof. E. de Robertis", Facultad de Medicina, Universidad de Buenos Aires, Argentina; [2]Center for Biomedical Research of La Rioja (CIBIR), Logroño, Spain; [3]Centro de Estudios Biomédicos, Biotecnológicos, Ambientales y Diagnóstico (CEBBAD), Universidad Maimónides, Buenos Aires, Argentina; [4]Department of Chemistry and Biochemistry, Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Madrid, Spain.
[¶]These authors contributed equally to this study and should be considered co-last authors.

Introduction. Perinatal asphyxia (PA) is responsible for a large proportion of neonatal deaths and numerous neurological sequelae, including visual dysfunction and blindness. During PA, the retina is exposed to ischemia/reoxygenation, which results in neuronal cell death and aberrant angiogenesis and gliosis. Since we have previously demonstrated that hypothermia prevents retinal damage caused by PA [1], we hypothesized that small molecule mimetics of hypothermia [2] may also prevent PA-induced retinal degeneration.
Materials and methods. Male rat pups were subjected to an experimental model of PA [1]. Four groups were studied: i) normally delivered (CTL); ii) normally delivered treated with 330 nmols/L zr17-2 (CTL-ZR); iii) exposed to PA for 20 min at 37ºC (PA); and iv) exposed to PA and, then, treated with zr17-2 (PA-ZR). Five days after birth, some rats were sacrificed and the eyes were studied by TUNEL assay. Forty five days after birth, other animals were subjected to electroretinography (ERG), sacrificed, and the eyes studied by histology,
Results. Electroretinography showed that PA animals had significant defects in the a- and b-waves and oscillatory potentials. The same animals presented a significant increase in the thickness of the inner retina and a large number of TUNEL-positive cells (Fig. 1). All these physiological and morphological parameters were significantly prevented by the treatment with zr17-2.
Conclusions. zr17-2 protects from cell death and restores electrophysiological function in the retina. This molecule could be used as a treatment to prevent the deleterious visual consequences of PA.

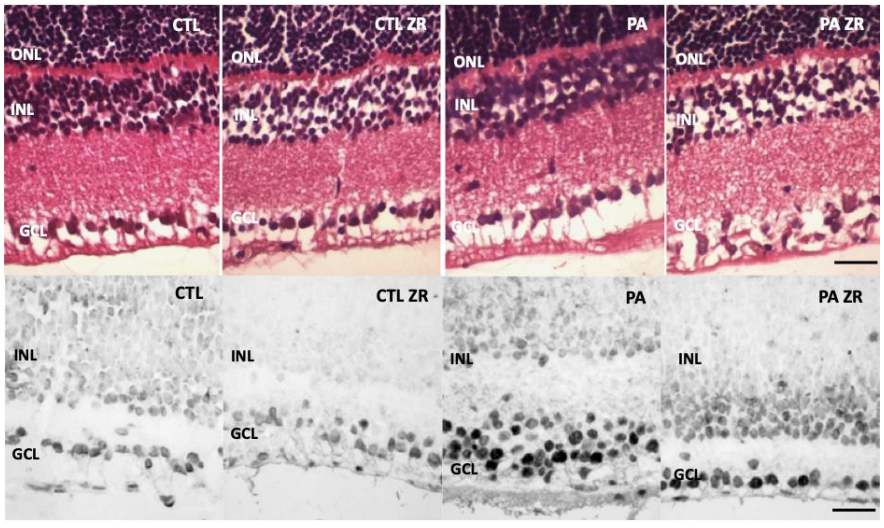


Fig. 1. Representative photographs of the retinas of animals belonging to the 4 experimental groups stained with H&E (upper row) or with TUNEL (lower row). ONL: outer nuclear layer; INL: inner nuclear layer; GCL: ganglion cell layer. Size bars = 25 µm.

[1] Rey-Funes M, et al. Front Pharmacol. 2021; 12: 651599
[2] Coderch C, et al. Bioorg & Med Chem. 2017; 25:6597-6604

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

TRPC calcium channels involved in death and survival of retinal cells

Caminos E.[1,2], Murillo-Martínez M.[1], García-Belando M.[1] and Martínez-Galán J.R.[1,2]

[1] Medical School of Albacete, Area of Histology. [2] Neurohistology and Development Group, Institute for Research in Neurological Disabilities (IDINE). [1,2] University of Castilla-La Mancha. Albacete, Spain

Background: Elevated intracellular calcium levels can initiate multiple detrimental pathways directly or via the endoplasmic reticulum which lend to cell death. Transient receptor potential canonical (TRPC) channels are a family of membrane calcium channels whose first member (TRPC1) has expression levels increased in neurodegenerative diseases such as Alzheimer or Parkinson, both diseases with manifestations into the retina. We considered the possibility that TRPC channels function as a store-operated calcium (SOC) channel in the retina, with special relevance during retinal neurodegeneration. Previous studies dated high calcium basal levels in glia and neuronal cells in the retina of P23H transgenic rats [1], an experimental model widely used to study retinosis pigmentaria (RP). RP is a group of inherited retinal dystrophies common in humans. We asked whether TRPC might be involved in the degeneration or in the cellular maintenance of P23H rat retinas where high calcium level could be a state of the retina resulting or causing degeneration.
Methods: Specific antibodies have been used to identify the cellular location of TRPC1 channel in healthy rat retina and to recognize changes during neurodegeneration in P23H-1 rats. Quantitative levels of TRPC1 protein expressed in control and degenerating retinas were measured by Western blot analysis. In addition, we identify functional interactions between TRPC family members by proximity ligation assays.
Results: Immunoreactivity showed TRPC1 to be prominently distributed across the inner retina with punctate labeling in horizontal, bipolar, amacrine and ganglion cell processes in the outer and inner plexiform layers; in specific bipolar and amacrine cell somata in the inner nuclear layer; and in cell bodies and fibers in the ganglion cell and optic nerve fiber layers. Curiously, TRPC1 expression was also found in intrinsically photosensitive retinal ganglion cells (ipRGC) but only in retinas with advanced degeneration. Müller cells and astrocytes showed an intense immunoreaction which increased with the progress of RP. TRPC1 protein levels were significantly higher in P23H retinas than in healthy retinas. In addition, we detected functional interactions between TRPC1 and TRPC5 channels mainly in the inner retina.
Discussion and Conclusions: These results demonstrate that TRPC1, alone or together with TRPC5, is present in healthy rat retinas and its expression changes in the inner retina during outer retina degeneration. TRPC1 may contribute to increase the cytoplasmic calcium levels in the retina through different pathways. It may work as a SOC channel in Müller cells and astrocytes where we also detected the presence of stromal interacting molecule 1 (STIM1), a member of SOC pathway. SOC signaling pathway could be also the source of cytosolic calcium in horizontal cell dendrites where there is calcium release from endoplasmic reticulum stores via ryanodine receptors [2]. In bipolar, specific amacrine and ganglion cells, TRPC1 must be activated via metabotropic and IP_3 receptors present in these cells. However, TRPC1 is not involved in the increase of cytoplasmic calcium in ipRGC in healthy retinas while it is present into these cells when the degeneration reaches the inner retina. The higher expression of TRPC1 during degeneration suggests that the channel could contribute to the cellular degeneration in these types of pathologies where calcium homeostasis is altered. Future studies will be necessary to better understand the role of TRPC1 in processes of degeneration and/or maintenance of retinal cells. The changes in TRPC1 expression found in this work suggest that TRPC channels could be targets to develop new therapies to control neurodegenerative diseases such as RP.

Supported by Junta de Comunidades de Castilla-La Mancha (FEDER; SBPLY/19/180501/000023) and Ayudas a Grupos de Investigación by UCLM.

[1] Caminos E., Vaquero, C.F. and Martinez-Galan J.R. (2015). Relationship between rat retinal degeneration and potassium channel KCNQ5 expression. Exp. Eye Res. 131, 1-11.
[2] Schubert T., Weiler R. and Feigenspan A. (2006). Intracellular calcium is regulated by different pathways in horizontal cells of the mouse retina. J. Neurophysiol. 96, 1278-1292.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Synaptic plasticity during retinal degeneration in the visual cortex of the rat

Martinez-Galan J.R.[1,2], Garcia-Belando M.[1], Cabanes-Sanchis J.J.[1] and Caminos E.[1,2]

[1]Facultad de Medicina, Universidad de Castilla-La Mancha, Albacete, Spain. [2]Grupo de Neurohistología y Desarrollo, Instituto de Investigación en Discapacidades Neurológicas, Universidad de Castilla-La Mancha, Albacete, Spain.

Introduction: The P23H mutant rhodopsin transgenic rat is an experimental model of retinal degeneration that exhibits gradual photoreceptor loss with similar properties as human autosomal dominant retinitis pigmentosa (RP). Interestingly, while the retina of these animals has been greatly studied, the rest of the central nervous systems remains poorly explored. The numerous advances in the repairing of the damaged retina in RP make necessary to know whether RP affects the structure of the visual cortex, because although a future therapeutic strategy could restore partly the damaged retina, it might not have any effect on the functional recovery of the vision. Loss of visual experience, induced by RP, increases the spontaneous firing rate of visual cortical neurons [1] suggesting the existence of possible morphological and/or molecular changes. However, such changes have not yet been described. Here, we are interested to find cortical alterations in young rats, when the progression of RP has affected only to a part of the retinal photoreceptor layer and later, in adulthood, when photoreceptor layer has almost disappeared, and retinal degeneration is more severe. To this goal, we focused on the study of the synaptic cytoarchitecture of the primary visual cortex (V1) by analyzing a series of pre- and postsynaptic parameters in relation to excitatory glutamatergic transmission.

Methods: Visual cortices from control Sprague Dawley (SD) and P23H rats were used at the postnatal days 30 (P30) and P230. At the presynaptic level, by using immunofluorescence, we have evaluated the distribution of the vesicular glutamate transporters (VGLUTs), namely VGLUT1, present in all the cortical layers, and VGLUT2, more specific of layer 4 thalamocortical synapsis. Postsynaptically, we have analyzed the expression of the postsynaptic density protein-95 (PSD-95) by using Western blot and the distribution of dendritic spines along apical shafts of layer 5 pyramidal neurons, stained by the Golgi-Cox method. Depending on the nature of the data, different types of tests were used to assess the differences between SD and P23H rats.

Results. The major findings of this study were: (1) We have observed that phenotypical alterations of the P23H rats include a decrease of the body weight, brain size and neocortical thickness as compared to SD rats; (2) in young rats, RP did not affect either pattern of distribution of presynaptic markers or the expression of PSD-95; (3) in adult rats, although no differences in VGLUT1 distribution or PSD-95 expression were found between both groups, an altered pattern of VGLUT2-immunoreactive thalamocortical terminals was observed in P23H rats, with stronger immunoreactivity confined to a narrower band in layer 4; (4) RP had no effect on the density and distribution of spines along apical shafts of pyramidal neurons in young rats, but significantly decreased the density of spines and altered their distribution in the adult P23H rats.

Discussion and conclusions: Although evident changes in the size of the P23H rat brains have been observed, cortical layering and cellular organization of V1 were preserved. During first stages of retinal degeneration (P30) the synaptic organization of the V1 was not affected probably due to the health of the central visual pathway probably maintained by the activity retinal ganglion cells. However, later stages of RP (P230) lead to an important decrease of sensory activity that generates more noticeable changes in some presynaptic (thalamocortical VGLUT2-immunoreactive terminals) and postsynaptic elements (dendritic spines from pyramidal layer 5) of the V1. Future studies will determine how reversible these changes are, after a hypothetical recovery of the damaged retina, and whether they affect the functionality of the visual system.

Supported by Junta de Comunidades de Castilla-La Mancha (FEDER; SBPLY/19/180501/000023) and Ayudas a Grupos de Investigación from UCLM.

[1] Wang Y., Chen K., Xu P., Ng TK. and Chan LLH. (2016). Spontaneous neural activity in the primary visual cortex of retinal degenerated rats. Neurosci. Letters 623,42-46.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Agenesis of corpus callosum as a consequence of a defect of the program of development in the neuroepithelium in a murine model of congenital hydrocephalus

Rodríguez-Pérez L.M.[1,3], Mercado Sáenz S.[1], Sánchez Varo R. [1], Escamilla Sánchez A.[1], Peláez González A.[1], Ríos Barranquero M.C.[1], Alba Tercedor M.C.[1], López Villodres J.A.[1], Jiménez A.J. [2,3] and Páez-Gonzalez P.[2,3]

[1]Department of Human Physiology, Human Histology, Anatomical Pathology and Physical Education. University of Malaga, 29010 Malaga, Spain; [2]Department of Cell Biology, Genetics and Physiology. University of Malaga, 29010 Malaga, Spain; [3] Biomedical Research Institute of Malaga (IBIMA), Spain

Introduction: Callosal agenesis is frequently associated to human cases of congenital hydrocephalus with interhemispheric cyst. The *hyh* mutant mice suffer a defect in the development of the neuroepithelium and congenital hydrocephalus with agenesis of corpus callosum.
Neuroepithelial-derived midline glial cells participate in the development of the corpus callosum, the glial wedge and the indusium griseum glial cells. Both populations secrete diffusible signals to form a path and guide the pioneering axons to cross the interhemispheric midline. The aim of this work is to clarify the relationship between the abnormal development of neuroepithelium taking place in congenital hydrocephalus and agenesis of corpus callosum.
Methods: Wild type (wt) and mutant hydrocephalic *hyh* mice from E13,5 to P1 were used to study the midline glial cell populations. RC2, nestin, GFAP and BLBP were used to characterize the neuroepithelium and the midline glial cells. Development of callosal axons was studied using DiI-Tracing in fixed brains and labelling with anti-NCAM and anti-GAP-43. Organotypic culture of brain slices with DiI-labelling was used to analyze *in vitro* the growth of pioneering axons towards the midline.
Results: in *hyh* mutant mice, ventriculomegaly was absent in lateral ventricles at all prenatal ages. An interhemispheric cyst is not present until E17,5 in the *hyh* mice. Pioneering axons are seen crossing the interhemispheric midline of wt mice at E15,5. At this age, hyh mice present a disruption of discrete areas of the ventricular zone in the cortico-septal border, which is the region where the glial wedge cells are originated. As consequence, these cells are not formed. Indusium griseum glial cells are present in both wt and mutant mice. In brain slices of hyh mice, DiI-Labelled pioneering axons are seen failing to find the lane to cross the midline and are wrongly directed towards the lateral ventricle. This direction taken by these axons can be explained as a consequence of the absence of chemorepellent signals from the glial wedge
Discussion & conclusions: Formation of corpus callosum takes place at stages where ventriculomegaly in the lateral ventricles and interhemispheric cyst are not present in the *hyh* mice. The absence of the glial wedge, as a consequence of a defective program of neuroepithelium development present in the *hyh* mice, can explain the agenesis of corpus callosum.

Supported by Junta de Andalucia (UMA18-FEDERJA-277) and Instituto de Salud Carlos III (PI19/00778), Spain; co-financed by FEDER funds from the European Union, Spain

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Staining techniques for the histological evaluation of myelin in native and regenerating nerve tissue

García-García O.D.[1,2], Weiss T.[3], El Soury M.[4,5], Chato-Astrain J.[1,2], Ávila-Fernández P.[1], Oyonarte S.[1,2,6], Crespo P.V.[1,2], García J.M.[1,2], Campos A.[1,2] and Carriel V.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Department of Plastic, Reconstructive and Aesthetic Surgery, Medical University of Vienna, Austria; [4] Nerve regeneration Group, Department of Clinical and Biological Sciences, University of Torino, Torino, Italy; [5] Neuroscience Institute Cavalieri Ottolenghi (NICO), University of Torino, Torino, Italy; [6] Network for Transfusional Medicine, Cells and Tissues, Granada, Spain

Introduction: Histological and, especially, histochemical analyses are basic tools in neurosciences to investigate the physiological and pathological state of the nervous system [1]. These techniques allow the identification of different tissue elements including myelin to establish a histopathological diagnosis. Moreover, these methods are valuable tools to evaluate degenerative or regenerative processes in experimental neural tissue engineering studies [2]. Myelin is composed of a complex group of lipids (such as proteolipids and lipoproteins) that can be identified using histochemical or immunohistochemical methods. The aim of this study was to evaluate the efficacy, sensibility, and morphological quality of the myelin staining in frozen sections and paraffin-embedded tissues.
Methods: Rat sciatic nerves were collected and processed as follows: i) direct freezing technique; ii) 4% neutral buffered formaldehyde fixation (24h), 20% sucrose cryoprotection (24h), and rapid freezing technique (by using cooled isopentane); iii) 4% neutral buffered formaldehyde fixation (24h), dehydration and conventional paraffin-embedding procedure; iv) 4% neutral buffered formaldehyde fixation (24h) followed by 1% osmium tetroxide (OsO_4) post-fixation and conventional paraffin-embedding. Histological sections were stained with luxol fast blue (LFB), MCOLL (LFB + picrosirius and hematoxylin), Sudan black (SB), FluoroMyelin™ fluorescent dye, and the myelin basic protein (MBP) and S-100 protein were co-stained by double immunofluorescence technique.
Results: The direct freezing and sectioning technique was considerably faster and more economic, but the histological quality was poorer, as expected. The combination of chemical fixation, cryoprotection, and fast freezing technique allowed to obtain high-quality cryosections suitable for histochemical, fluorescence, and immunofluorescence techniques. In relation to the staining used, all of them were able to stain myelin. MCOLL provided considerably more information than the other staining used as it also specifically stains the collagen network and cell nuclei. OsO4 method permitted to obtain specific and high-resolution myelin images without the use of contrast, being a fast and easy method. Sudan black, FluoroMyelinTM and MBP/S100 immunofluorescence techniques were optimal when the nerve samples were chemically fixed, cryoprotected, frozen.
Discussion: A correct selection of the tissue fixation and processing technique is crucial for the experimental design. To evaluate the myelin content, different tissue-processing and staining techniques can be performed. The four methods evaluated here provided highly specific and good-quality images of the myelin content. For FluoroMyelin™ and MBP/S100 immunofluorescence techniques chemically fixed, cryoprotected and cryosections are recommended. In light microscopy studies, better results can be obtained with MCOLL, especially in paraffin-embedded material. This study provides four methods to accurately identify myelin that could serve as histological quality controls in different experimental contexts, especially in neural tissue engineering [2].

Supported by the Spanish Plan Nacional de Investigación Cientifica, Desarrollo e Innovación Tecnológica (I+D+i) of the Spanish Ministry of Science and Innovation, grants FIS PI14/1343, FIS PI17/0393 and FIS PI20/0318, Instituto de Salud Carlos III. Supported by Plan Andaluz de Investigación, Desarrollo e Innovación (PAIDI 2020), Consejería de Transformación Económica, Industria, Conocimiento y Universidades, Junta de Andalucía, Spain, grants P18-RT-5059 and A-CTS-498-UGR18 (proyectos de I+D+i en el marco del Programa Operativo FEDER Andalucía 2014-2020, University of Granada). Co-financed by the European Regional Development Fund (FEDER - ERDF).

[1] Chato-Astrain J, et al. (2021) Peripheral Nerve Tissue Engineering and Regeneration (book). Series in Biomedical Engineering, Springer. doi.org/10.1007/978-3-030-21052-6_14
[2] García-García OD, et al. (2022). Histochemistry of single molecules (book). 2ed Series Methods in Molecular Biology, Springer

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

The hypothermia mimetic molecule zr17-2 prevents retinal lesions in a model of experimental compression of the optic nerve

Rey-Funes M.[1], Contartese D.S.[1,2], Peláez R.[3], Soliño M.[1], Fernández J.C.[1], Ciranna N.S.[1], Nakamura R.[1], Sarotto A.[1], Dorfman V.B.[4], Zapico J.M.[5], Ramos A.[5], de Pascual-Teresa B.[5], López-Costa J.J.[1], Larrayoz I.M.[3], Loidl C.F.[1,¶] and Martínez A.[3,¶]

[1]Instituto de Biología Celular y Neurociencia "Prof. E. de Robertis", Facultad de Medicina, Universidad de Buenos Aires, Argentina; [2]División de Oftalmología, Hospital de Clínicas "José de San Martín", Facultad de Medicina, Universidad de Buenos Aires, Argentina; [3]Center for Biomedical Research of La Rioja (CIBIR), Logroño, Spain; [4]Centro de Estudios Biomédicos Básicos, Aplicados y Desarrollo (CEBBAD), Universidad Maimónides, Buenos Aires, Argentina; [5]Department of Chemistry and Biochemistry, Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Madrid, Spain.
[¶]These authors contributed equally to this study and should be considered co-last authors.

Injuries to the optic nerve (ON) are a common occurrence that produces anterograde and retrograde axonal degeneration, with death of retinal ganglion cells and vision loss. Previously, we showed that ocular temperature reduction after experimental compression of the ON (intraorbital optic nerve crush, IONC) significantly diminishes vision loss, an effect probably mediated by cold inducible RNA-binding protein (CIRP) [1]. The synthetic molecule zr17-2 is a hypothermia mimetic able to prevent CIRP degradation in cultured cells [2]. Our aim was to evaluate the effect of zr17-2 on retinal lesions due to ON trauma. Adult male rats (n=28) were randomly distributed into 4 experimental groups: i) sham surgery with intravitreal injection of vehicle (CTL V); ii) sham surgery with intravitreal injection of 5 µl of 330 nmols/L zr17-2 (CTL ZR); iii) IONC surgery with intravitreal injection of vehicle (IONC); and iv) IONC surgery with intravitreal injection of zr17-2 (IONC ZR). Some animals were sacrificed 5 days after surgery and eyes processed for TUNEL. Others were subjected to electroretinography (ERG) 15 days after surgery. For ERG, animals were kept in the dark for 12 h and inspected for scotopic ERG and oscillatory potentials (OP) to evaluate the integrity of the visual pathway.

Retinas of the CTL V and CTL ZR groups showed very few TUNEL-positive cells (Fig.1). In contrast, retinas of the IONC group had a significantly high number of apoptotic cells ($p<0.0001$). Treatment with zr17-2 significantly reduced the number of TUNEL positive cells induced by IONC ($p<0.0001$), although this treatment was not enough to prevent all the damage (Fig. 1).

Regarding the ERG experiment, eyes in the IONC group displayed significantly lower a-wave, b-wave, and OP amplitude than the controls. The IONC ZR eyes showed a significant prevention of the ERG signals ($p<0.05$), although they did not reach the control levels.

In conclusion, the present work indicates the effectiveness of zr17-2 *in vivo* to prevent retinal lesions constituting an innovative treatment for ON trauma, and enabling its use in future preclinical trials.

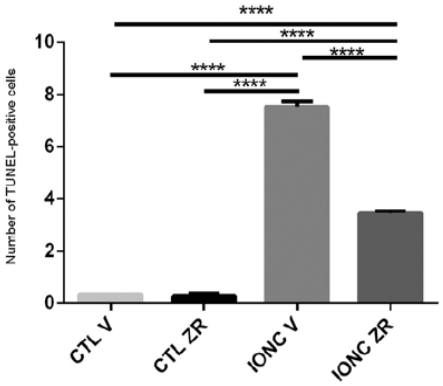


Fig. 1. Quantification of TUNEL-positive cells in the ganglion cell layer. Each bar represents the mean ± SEM of 10 animals and 6 microscopic fields per animal. ****: $p<0.0001$

[1] Rey-Funes M, et al. Scientific Reports. 2017; 7:6966
[2] Coderch C, et al. Bioorg & Med Chem. 2017; 25:6597-6604

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Oligodendrocyte metabolism throughout its differentiation: immunocytochemistry study and its impact in remyelination

Gismero Rodríguez J.[1], López Villodres J.A.[1], Escamilla Sánchez A.[1], García Díaz B.[2], Rodríguez Pérez L.M.[1], Mercado Sáenz S.[1], Ortega Jiménez M.V.[1,3], Arranz Salas I.[1,3], Peláez González A.[1] and Bermúdez Flores D.[1]

[1] Department of Human Physiology, Human Histology, Anatomical Pathology and Physical Education. University of Malaga (UMA), 29010. Malaga, Spain; [2] Biomedical Research Institute of Malaga (IBIMA), Spain; [3] Unit of Anatomical Pathology, Virgen de la Victoria University Hospital, 29010 Malaga, Spain

Introduction: Oligodendrocytes (OL) role in demyelinating pathologies such as multiple sclerosis and other neurodegenerative diseases is only recently being subject of extensive research. While the genetic and molecular aspects have been thoroughly studied, their metabolism was overshadowed. In order to develop new therapies to promote remyelination of already damaged axons, we need to accurately describe how OL metabolism affects axon myelination and trophic support (1). The objective of this study is to obtain cytological evidence of the extent of both glycolytic metabolism and oxidative phosphorylation by immunocytochemistry throughout the development of OL.
Methods: Oligodendroglia cells from post-natal mice cortices were obtained and cultured. A wide assortment of differentiation-stage-specific cell surface antigens, a glycolytic and an oxidative phosphorylation marker were combined in several immunofluorescences to study both metabolic pathways in each step of differentiation.
Results: After analysing them under confocal microscopy and imaging software, we observed a constant upregulation of glycolytic metabolism throughout differentiation, while oxidative phosphorylation seemed to increase with differentiation to then decrease when oligodendrocytes achieved their final maturation stage.
Conclusions: Therefore, oxidative phosphorylation may be crucial in the differentiation of precursors and glycolysis would thus be the preferred metabolic pathway for fully matured OL.

[1] Rosko L. et al. Neuroscientist. 2019;25(4):334–43.

Supported by UMA and IBIMA and funding from two ongoing projects: - ‘Modulation of oligodendrocyte metabolism via blood vessel remodelling as target to promote remyelination’ (funding by NEURATRIS). - ‘Blood vessel remodelling modulates remyelination by oligodendrocyte metabolic reprogramming’ (funding by Arsep Foundation).

SESSION

REPRODUCTION AND FERTILITY AND HISTOLOGICAL TEHCNIQUE

REPRODUCCIÓN Y FERTILIDAD Y TÉCNICA HISTOLÓGICA

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Differential distribution of alfa and beta-adrenergic receptors related to human sperm functional regions

Girela JL.[1,2], De Juan A.[2,4], Simó E.[1], Grande E.[1] and De Juan J.[2,3]

[1] Department of Biotechnology, [2] Biotechnology Research Group, [3] IUEG, University of Alicante, Alicante, Spain. [4] San Juan University Hospital, Alicante, Spain

Introduction: Human spermatozoa are terminally differentiated cells that present regionalization with clearly identifiable structures (i.e., acrosome region, midpiece and terminal piece of the flagellum). It is known that each of these parts plays a different role in the fertilization process. The control of these processes is regulated by neuronal receptors and ion channels associated with them, among which we can find members of the adrenergic receptors family such as alfa 2a (ADRA2a) and beta 2 (ADRB2) adrenergic receptors. The main objective of this study is to determine the presence and distribution of those receptors in human spermatozoa.
Methods: After written consent and ethical approval, semen samples from 5 young, healthy volunteers were used. All the samples were considered comparable to the reference population according to WHO 2021 criteria for semen analysis. As a positive control, we used the neuroblastoma cell line SH-SY5Y known to express both receptors ADRA2a and ADRB2. Immunofluorescence (IF) techniques were performed to localize the receptors. In this study, we validate the use of an anti-ADRB2 primary monoclonal antibody produced in mouse (Abcam, UK) to be used for IF, as it has only been used for Western Blot techniques previously. For the ADRA2a we used an anti-ADRA2a primary polyclonal antibody produced in rabbit (Abcam, UK). As secondary antibodies, we used an anti-mouse IgG or anti-rabbit IgG fluorochrome-conjugated secondary antibody (Jackson Laboratories, USA). Samples were fixed in 4% paraformaldehyde for 5 minutes before depositing them on a poly-L-lysine solution coated cover-slide. A blocking solution containing 5% BSA and 2% FCS was used. Negative controls were performed in immunolabelling techniques by omitting the primary antibody. Patterns of distribution of the receptors were determined by Laser Scanning Confocal (LSC) microscopy. The frequencies of the different patterns were quantified by counting almost 200 sperm cells of each sample, and differences were assessed using the ANOVA test followed by a posteriori Bonferroni Test.
Results: The specificity and validity for the use in IF applications of the anti-ADRB2 antibody were demonstrated, in both neuroblastoma and sperm cells. In the case of ADRB2, four patterns of distribution were identified. Of all of them, the most frequent and the only one that shows statistical differences (ANOVA and Bonferroni test $p<0,01$) was the one with labelling on the midpiece and the terminal piece of the flagellum. The rest of the patterns can be associated with immature states of the spermatozoa present in the sample. For the ADRA2a, two different patterns were observed. The most frequent and consistent with those sperm cells that show a normal morphology was restricted to the acrosome region of the head and the principal piece of the flagellum. Those sperm with cytoplasmic droplets showed the alternative pattern with strong labelling of the midpiece.
Discussion & conclusions: We observed a differential localization of the receptors studied in functional regions of the spermatozoa. It has been suggested that ADRA2a is related to the control of the acrosome reaction, which is consistent with its presence in the acrosomal region. ADRB2 is believed to control sperm motility, which is in concordance with the observed distribution in the middle and terminal piece of the flagellum. In conclusion, the differential distribution of the studied receptors seems to be related to suggested functions that they perform on the physiology of the human spermatozoa.

Supported by UAUSTI20-07 and UAUSTI21-08 from the University of Alicante.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

HSPA2 expression on unbound and bound mouse spermatozoa during in vitro fertilization

Huerta-Retamal N.[1], Sáez-Espinosa P.[1], Robles-Gómez L.[1], Aizpurua J.[2,3], Bermejo-Álvarez P.[4] and Gómez-Torres M.J.[1,2]

[1] Departamento de Biotecnología, Universidad de Alicante, Alicante, España; [2] Cátedra Human Fertility, Universidad de Alicante, Alicante, España; [3] IVF SPAIN, Reproductive Medicine, Alicante, España; [4]Animal Reproduction Department, INIA, CSIC, Madrid, España.

Introduction: The importance of heat shock protein A2 (HSPA2) in reproduction is evident since its ablation causes infertility in males. Animal models, such as mice, constitute a valuable resource to circumvent the ethical and technical limitations associated to human IVF. The objective of this study was to identify the localization of HSPA2 in mouse spermatozoa according to their ability to bind to the zona pellucida (ZP) during *in vitro* fertilization.

Methods: Oocytes obtained from superovulated CBAXC57BL6 F1. females were incubated with spermatozoa obtained from CBAXC57BL6 F1 hybrid males of proven fertility. After 2h of incubation, sperm bound to the ZP or unbound (free-swimming sperm) were recovered and fixed. The distribution of HSPA2 was assessed by indirect immunofluorescence using anti-HSPA2 antibody. A minimum of 200 sperm per group were analysed under confocal microscopy. Statistical differences were tested by t-test ($p < 0.05$).

Results: HSPA2 was detected in 85.5% of sperm failing to bind ZP and 96.1% in ZP-bound sperm. Sperm displayed 4 different HSPA2 distribution patterns: pattern 1 (P1) intense fluorescence in the upper third of the acrosome and the equatorial segment; pattern 2 (P2), concentrated fluorescence in the post-acrosomal region; pattern 3 (P3), weak labelling in the equatorial segment accompanied by intense fluorescence in the acrosome; and pattern 4 (P4) fluorescence in the posterior segment of the acrosome and the ventral region. Sperm unable to bind ZP were highly heterogeneous for these patterns (**table 1**), showing roughly equal proportions of each pattern (P1 28.7%, P2 26.9 %, P3 25.1 % and P4 19.3%). In contrast, in ZP-bound sperm the significantly most representative patterns were P2 (36.7%) and P4 (34.5%) (**table 1**), showing a significative increase compared to unbound cells.

Discussion & conclusions: Our data suggest that HSPA2 is differently located according to the ability of sperm cells to bind to ZP. To properly fulfil its role during fertilization, this chaperone must be located in the post-acrosomal region and the ventral region, pointing HSPA2 to be a promising candidate for use as fertility biomarker.

Supported by VIGROB-186 and Human Fertility Chair of the University of Alicante.

Table 1: Mean (%) ± standard deviation of cells with immunofluorescence for HSPA2 and frequency of HSPA2 patterns in mouse sperm cells that were positive for HSPA2 in the different experimental conditions. *p<0.05 against not bound sperm

		HSPA2 distribution patterns			
	Positive cells for HSPA2 (%)	P1 (%)	P2 (%)	P3 (%)	P4 (%)
Not bound to ZP	85.5 ± 3.4	28.7 ± 6.8	12.4 ± 3.3	25.1 ± 8.4	19.3 ± 6.0
Bound to ZP	96.1 ± 0.8*	6.7 ± 1.4*	36.7 ± 2.6*	18.3 ± 2.6	34.5 ± 2.9*

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Influence of vitrification and trichostantin A on chromatin configuration and H3K9 acetylation in human germinal vesicles

Sáez-Espinosa P.[1], Gimeno-Camps C.[2], Moya I.[2], Torres P.[2], Peinado I.[2] and Gómez-Torres M.J.[1]

[1] Departamento de Biotecnología, Facultad de Ciencias, Universidad de Alicante, Alicante, Spain; [2] Unidad de Reproducción Asistida Humana, Hospital Universitario y Politécnico La Fe, Valencia, Spain.

Background: Different morphological and molecular markers have been proposed to predict the meiotic and developmental capacity of antral oocytes. One of these indicators is the chromatin organization of the germinal vesicle (GV). It is known that non-surrounded nucleolus oocytes (NSN, dispersed chromatin) remain transcriptionally active, while surrounded nucleolus oocytes (SN, chromatin condensed around the nucleolus) are transcriptionally inactive. Another parameter is the degree of dynamic post-translational modifications, including acetylation, methylation, and phosphorylation, at specific residues on N-terminal histone tails. Due to the importance of chromatin organization and dynamic epigenetic changes for proper chromosome segregation, in this research, we have combined the analysis of chromatin configuration with the detection of histone H3 lysine 9 acetylation (H3K9ac) in human GVs.

Methods: A total of 157 GVs from patients attended at the Unidad de Reproducción Asistida Humana of the Hospital Universitario y Politécnico La Fe (Valencia, Spain) were decumulated and divided in three experimental conditions: 54 GVs were fixed directly (Control), 54 GVs were vitrified and thawed (Vit), and 49 GVs were exposed to 30nM of trichostantin A (TSA) for 1h. All the oocytes from different groups were fixed in 2% paraformaldehyde. After that, oocytes were incubated overnight with a primary H3K9ac antibody (1:200) produced in rabbit. After that, the cells were incubated with the secondary antibody anti-rabbit conjugated with Cyanine™3 (1:300) for 90min. For chromatin staining, we used DAPI. This experimental part was performed at the Departamento de Biotecnología of the Universidad de Alicante (Alicante, Spain). Finally, the oocytes were analyzed by Confocal Laser Scanning Zeiss LSM 800 Microscope and the statistical analysis was performed by IBM SPSS Statistics 19.0.

Results: In relation to the chromatin organization, we identified seven patterns: non-surrounded nucleolus (NSN), partly non-surrounded nucleolus (pNSN), prematurely condensed non-surrounded nucleolus (cpNSN), prematurely condensed surrounded nucleolus (cpSN), partly surrounded nucleolus (pSN), surrounded nucleolus (SN), and germinal vesicle breakdown (GVBD). Specifically, regarding the configuration of chromatin, it should be noted that the majority pattern observed in each group was significantly different (P<0.05). In Control, the SN configuration was mostly observed; in Vit condition, the predominant was GVBD; and in TSA group, the majority was cpNSN organization (See Figure 1). In terms of acetylation, the presence of H3K9ac was observed in all experimental conditions and its location was correlated with the chromatin distribution.

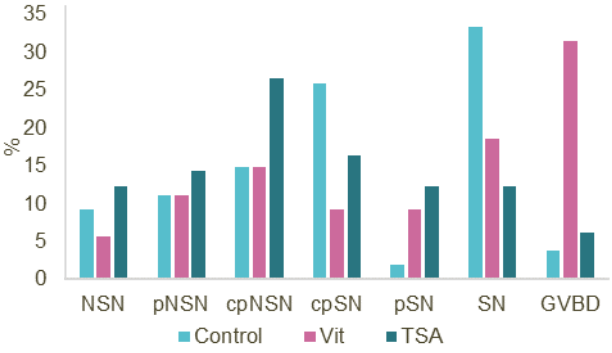


Figure 1. Chromatin distribution percentages in human GVs from each experimental condition.

Discussion and conclusions: Our findings reported that compared with control, vitrification and TSA exposure alters H3K9 acetylation, with subsequent chromatin distribution modifications in human GVs. Since vitrification leads to an increase in GVBD and therefore to premature compaction of chromosomes, this could be affecting the epigenetic state of GVs, and at least in part, explain the lower developmental competence of vitrified/thawed oocytes. For its part, the inhibition of histone deacetylases (HDAC) with TSA revealed that HDACs are essential for chromatin remodeling in GVs. Overall, our results provide new insights into the epigenetic bases related to chromatin arrangement and could contribute to the improvement of gamete cryopreservation techniques.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

IZUMO1 and TMEM95 relocalization after spontaneous acrosome reaction in rabbit sperm

López-Botella A.[1,*], Sáez-Espinosa P.[1,*], Balastegui-Alarcón M.[2], Hernández-Falcó M.[1], Huertas-Retamal N.[1], Avilés M.[2] and Gómez-Torres M.J.[1]

[1] Departamento de Biotecnología, Facultad de Ciencias, Universidad de Alicante, 03690 Alicante, Spain; [2] Departamento de Biología Celular e Histología, Facultad de Medicina, Universidad de Murcia, IMIB-Arrixaca 30100 Murcia, Spain; * These authors contributed equally to this work.

Introduction: The proper adhesion and fusion of gamete membranes during fertilization is an essential process for sexual reproduction. It has been shown that IZUMO1 and TMEM95 (Transmembrane Protein 95) proteins are critical along sperm-oocyte fusion in some mammal species. Due to the importance of these proteins, here, we have described the redistribution of IZUMO1 and TMEM95 after spontaneous acrosome reaction in rabbit sperm.

Methods: Seminal samples of rabbits were obtained by expert staff from the animal facility of the University of Murcia (Murcia, Spain). Then, sperm were fixed in 2% paraformaldehyde-PBS during 1h at 4ºC. For the detection of IZUMO1/TMEM95 and the spontaneous acrosome reaction a double labeling was performed. First, sperm were incubated with the primary polyclonal anti-IZUMO1 (Biorbyt Ltd) or anti-TMEM95 (MyBioSource) antibodies produced in rabbit (1:100) overnight. Following, cells were incubated with a secondary antibody anti-rabbit IgG conjugated with FITC (1:100) for 1h. Then, acrosome status was labelled using the lectin PNA-FITC (50 µg/mL, Vector Laboratories) for 45 min. Finally, samples were mounted using Vectashield medium containing DAPI. Appropriate negative controls were performed to confirm that there was no labeling in the sperm, and cells were evaluated using LEICA DM fluorescence microscope.

Results: In this study, we evidenced the presence of IZUMO1 and TMEM95 in rabbit sperm and their relocalization after spontaneous acrosome reaction. Considering IZUMO1 immunostaining, in acrosome-intact sperm the labelling was present in the acrosomal and postacrosomal region with a higher intensity in the equatorial segment. Nevertheless, acrosome-reacted cells showed a faint labeling in the postacrosomal region (Figure 1).

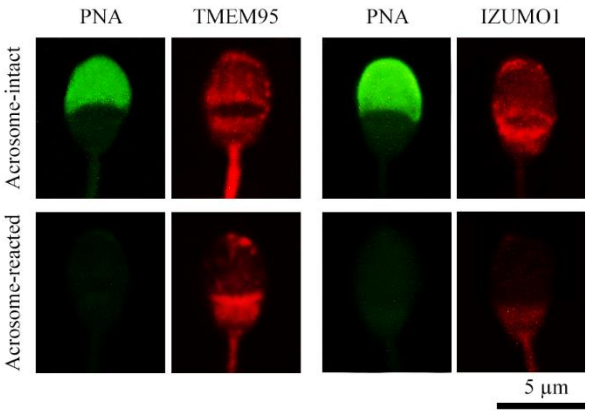


Figure 1. Acrosomal status-dependent localization of IZUMO1 and TMEM95 in rabbit sperm.

Regarding TMEM95 immunolocalization, our results showed that this protein was positioned at acrosomal and post-acrosomal region. However, in acrosome-reacted cells, TMEM95 relocated mainly to the equatorial segment and postacrosomal region (Figure 1).

Discussion and conclusion: The available research have highlighted the importance of IZUMO1 relocalization from the acrosome to the equatorial segment and postacrosomal region during the acrosomal reaction in several species. Our results coincide with previous studies and highlight the involvement of IZUMO1 in the sperm-oocyte fusion process after the acrosome reaction. Regarding TMEM95 immunolocalization, although little information is available, it is worth mentioning that this protein is found in the outer acrosomal membrane of mouse sperm and is relocated to the equatorial segment after the acrosome reaction. Related to this, our results also recorded the relocalization of TMEM95 to the equatorial segment and postacrosomal region in rabbit acrosome-reacted sperm. Therefore, IZUMO1 and TMEM95 localization in rabbit sperm (Figure 1) could be indicating that these proteins perform their main function at different stages of the fertilization process. Overall, our results provide new insights in the molecular basis of sperm fusion-required proteins and could contribute to the improvement of sperm selection techniques. However, additional research is necessary to deeply understand these proteins redistribution during sperm capacitation.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

ZP2 protein is not essential for ZP formation, folliculogenesis and fertility in rabbit (*Oryctolagus cuniculus*)

Sòria-Monzó P.[1], Balastegui-Alarcón M.[1], Cots-Rodríguez P.[1], Ballesta J. [1], Vicente Antón J.S.[2], Marco-Jiménez F.[2], Bermejo-Álvarez P.[3], Sáez-Espinosa P.[4], Gómez-Torres M.J.[4], Izquierdo-Rico M.J.[1] and Avilés M.[1]

[1] Department of Cell Biology and Histology, Faculty of Medicine, Universidad de Murcia and IMIB-Arrixaca, Murcia, Spain; [2] Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, Valencia, Spain; [3] Animal Reproduction Department, INIA, Madrid, Spain; [4] Biotechnology Department, Faculty of Sciences, University of Alicante, San Vicente del Raspeig, Alicante, Spain.

The zona pellucida (ZP) is an extracellular glycoproteic matrix synthesized at the ovary during follicular development and it has a key role in several mammalian reproductive processes such as oogenesis, fertilization, and preimplantation development. ZP is specie-specifically composed by three to four different glycoproteins (ZP1, ZP2, ZP3 and ZP4) in eutherian mammals. Thus, mouse ZP (*Mus musculus*) lacks ZP4 while both human and rabbit ZP (*Oryctolagus cuniculus*) have these four proteins. Genetic ablation of ZP2 (*ZP2*-KO) in mice produced oocytes lacking a ZP, showing an impairment in folliculogenesis, fertility and development [1]. Moreover, a single domain in the N-terminal portion of this protein is involved in the sperm binding in both mice and humans [2,3]. Premature STOP codon mutations found in infertile women patients trigger the formation of thinner zona pellucida and a defective sperm-binding [4,5]. The aim of this study was to evaluate the role of ZP2 on zona pellucida formation and fertility using a *ZP2*-KO rabbit model due to the similarity between rabbit and human ZP. We have produced a novel *ZP2*-KO rabbit model using CRISPR-Cas9 technology following our own previous reported protocols [6]. A one-nucleotide insertion mutation localized in the exon 3 of genomic rabbit *ZP2* has been achieved, which changes the reading frame and triggers a premature STOP codon in the 72 nt of the ORF. Rabbits with this mutation have been cross-bred to finally obtain a biallelic *ZP2*-KO. For the folliculogenesis study, ovarian samples of *ZP2*-KO female rabbits were obtained post-mortem, fixed with 4% formol and embedded in paraffin according to routine protocol. Then, 5 µm thick slides were sectioned and stained with Hematoxylin and Eosin and scanned using the Digital Slide Scanner Pannoramic MIDI II (3DHistech). Different preantral and antral ovarian follicles were observed, and no histological differences were found between KO and WT animals. An apparently intact ZP was observed surrounding the oocyte of the different ovarian follicles. To assess the fertility of *ZP2*-KO females, 3 females were mated twice with fertility probed males. All females gave birth at least once, having a total of 5 out of 6 possible deliveries (83.3%). The average litter size was 8.62±1.82, showing no differences in comparison to WT (T-Student $p>0.05$). In conclusion, we have demonstrated that ZP2 is not compulsory for the ZP formation, folliculogenesis and fertility. These results strongly support that ZP2 is not responsible of the sperm binding to the ZP in rabbit. Moreover, these results differ from both human and mouse, standing out the species-specific role of the ZP proteins.

This research was supported by the Spanish Ministry of Science and Innovation (Grant PGC2018-094781-B-I00) (MCINN/AEI/FEDER, UE).

[1] Rankin T.L., O'Brien M., Lee E., Wigglesworth K., Eppig J. and Dean J. (2001). Defective zonae pellucidae in Zp2-null mice disrupt folliculogenesis, fertility and development. Develop. 128, 1119–1126.
[2] Baibakov B., Boggs N.A., Yauger B., Baibakov G. and Dean J. (2012). Human sperm bind to the N-terminal domain of ZP2 in humanized zonae pellucidae in transgenic mice. J. Cell Biol. 197, 897–905.
[3] Avella M.A., Baibakov B. and Dean J. (2014). A single domain of the ZP2 zona pellucida protein mediates gamete recognition in mice and humans. J. Cell Biol. 205, 801–809.
[4] Dai C., Hu L., Gong F., Tan Y., Cai S., Zhang S., Dai J., Lu C., Chen J., Chen Y., Lu G., Du J. and Lin G. (2019). ZP2 pathogenic variants cause in vitro fertilization failure and female infertility. Genet. Med. 21, 431–440.
[5] Luo G., Zhu L., Liu Z., Yang X., Xi Q., Li Z., Duan J., Jin L. and Zhang X. (2020). Novel mutations in ZP1 and ZP2 cause primary infertility due to empty follicle syndrome and abnormal zona pellucida. J. Assist. Reprod. Genet. 37, 2853–2860.
[6] Lamas-Toranzo I., Fonseca Balvís N., Querejeta-Fernández A., Izquierdo-Rico M.J., González-Brusi L., Lorenzo P.L., García-Rebollar P., Avilés M. and Bermejo-álvarez P. (2019). Zp4 confers structural properties to the zona pellucida essential for embryo development. Elife 8, 1–18.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Distribution of Dopamine Type 2 Receptors (D2R) in human sperm surface, studied with Field Emission Scanning Electron Microscope (FE-SEM) and Graph-based methods

De Juan A.[1], Girela J.L.[1] and De Juan J.[1,2]

1Biotechnology Research Group, Department of Biotechnology, Alicante University, Spain, [2]IUIEG, Alicante University.

Introduction: Dopamine (DA) is a neuromodulator of the Central Nervous System (CNS), and its receptors (DRs) are distributed throughout the organism, modulating several activities such as motor functions, motivations, drive, and cognition [1]. DA is present in high concentrations in semen and oviductal fluid in the mammalians. In sperm, dopamine receptors (DRs) also have been detected, suggesting that DRs control important reproductive functions in humans, improving sperm motility and viability in animal models [2]. For some authors, DA intervenes in the reproductive behaviour of mammals, although this participation is controversial [3]. The recent identification of the Dopamine Receptor 2 (D2R) in sperm has promoted investigations about the effects of agonists and antagonists of D2R on their functions [4, 5, 6]. The main goal of this work is to develop a new approach to analyze the distribution of the D2R in human spermatozoa, using FE-SEM and Graph-theoretic analysis as tools.

Materials and methods: Sperm samples from 15 young, healthy volunteers were used. Normozoospermic samples were selected and processed following the WHO 2010 criteria. The D2R were immunolabeled with a primary monoclonal antibody (Anti-D2R, Abcam) followed by a 12 nm gold conjugated secondary antibody. The gold conjugated particles (GCPs) corresponding to D2R were studied using FE-SEM. Their images were processed to obtain the following data: (1) The number and location of the GCPs, (2) the distribution patterns of the GCPs on the spermatozoon surface, and (3) Graph-theory analysis parameters such as Nearest Neighbourhood distance (NN) and its Regularity Index (NNRI), Voronoi Domain area (VD) and its Regularity Index (VDRI) and those related to the Density Recovery Profile (DRP).

Results and conclusions: Using FE-SEM, the D2Rs in sperm show a number of GCPs (65.90 ± 10.47/μm2) much higher than the average GCPs observed in the background (0.71 ± 0.45/μm2). Although GCPs can be observed in all anatomical areas of human spermatozoa, their maximum expression takes place in the head (91.87%), with the most significant number of them (86.15%) located in the posterior band (PB), being very scarce or absent in the rest of locations. The Graph-theory analysis parameters showed the following data about the GCPs distribution in the PB: 1) The normalized NNRI is <1, which indicates a clustered pattern, 2) The normalized VDRI is <1, which shows a high overdispersion and 3) DRP elements values point to distribution in conglomerates.

[1] Martel, J.C. and McArthur, S.G., (2020). Dopamine Receptor Subtypes, Physiology and Pharmacology: New Ligands and Concepts in Schizophrenia. Front. Pharmacol., 14 July 2020; https://doi.org/10.3389/fphar.2020.01003.

[2] De Angelis, C., Cariati, F., Galdiero, G. Coppola, G., Galdiero, M., Salzano, C., Pivonello, C., Patalano, R., Alviggi, C., De Placido, G., Colao, A. Colao and, Pivonello, R. (2016). The role of dopamine pathway on human sperm: in vitro effect of dopamine receptor agonists and antagonists on sperm motility, kinetics and viability. Endocrine Abstracts 41 EP712 | DOI: 10.1530/endoabs.41.EP712

[3] Urra J.A.,Villaroel-Espíndola, F., Covarrubias, A.A., Rodríguez-Gil, J.E., Ramírez-Reveco, A., Concha, I.I. (2014). Presence and Function of Dopamine Transporter (DAT) in Stallion Sperm: Dopamine Modulates Sperm Motility and Acrosomal Integrity, Plos One. https://doi.org/10.1371/journal.pone.0112834

[4] Seeman, M.V. (2021). History of the dopamine hypothesis of antipsychotic action. World J Psychiatry. 11(7): 355–364.

[5] Bonifazi, A., Yano , H., Guerrero, A.M., Kumar, V., Hoffman, A.F., Lupica, C.R., Lei Shi, Newman, A.H. ACS Pharmacol Transl Sci. (2019) Feb 8;2(1):52-65. Presence and Function of Dopamine Transporter (DAT) in Stallion Sperm: Dopamine Modulates Sperm Motility and Acrosomal Integrity.

[6] Madrás, B. (2013). History of the Discovery of the Antipsychotic Dopamine D2 Receptor: A Basis for the Dopamine Hypothesis of Schizophrenia. Journal of the History of the Neurosciences 22: 62-78.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Analysis of new potential biomarkers in progressive skeletal muscle aging: application of liquid biopsy in the monitoring of sarcopenia

Fernández-Lázaro D.[1,2], Garrosa E.[3] and Garrosa M.[2,3]

[1]Department of Cell Biology, Histology and Pharmacology, Faculty of Health Sciences, University of Valladolid, Campus de Soria, Spain; [2]Research Group in Neurobiology, Faculty of Medicine, University of Valladolid, Spain; [3]Department of Cell Biology, Histology and Pharmacology, and INCYL, University of Valladolid, Valladolid, Spain

Background: Sarcopenia (Sp) is the loss of skeletal muscle mass associated with aging, causing alterations in the homeostasis of proteins, hormones, and mitochondria, which trigger an involution of muscle function and strength. Satellite cells (Sc) are myogenic stem cells, which are activated by injury or stress, and repair muscle tissue. With advancing age, there is a decrease in the efficiency of the regenerative response of Sc [1]. The set of Sc activities, such as quiescence, activation, self-renewal, and differentiation is coordinated by the interaction between pathways and niche signals. Therefore, the Sc aging process would be a consequence of the combined effects of age-dependent environmental alterations and Sc-associated intrinsic dysregulations [2]. Diagnosis of Sp occurs by direct assessment of muscle [1]; however, the detection of biomarkers in real-time biofluids by liquid biopsy could represent a step-change in the understanding of the molecular biology and heterogeneity of Sp since this noninvasive technique, useful for prognostic and predictive purposes, is capable of overcoming the limitations of tissue biopsies [3], (Figure 1). Therefore, we analyzed the scientific literature on the most influential biomarkers in Sp biologically and/or functionally related to Sc. **Methods:** We performed a narrative literature review with a search until May 2022 in the following electronic databases: Medline (PubMed), SciELO, and Cochrane Library Plus. Among all the records identified, 37 studies met the inclusion/exclusion criteria and provided data of interest on the objective of the study. **Results:** We identified a total of 13 potential biomarkers of Sp by their physiological and biological interaction with Sc. **Discussion:** We report that expression of GDF11, PGC-1α, Sirt1, Pax7, Pax3, Myf5, MyoD, CD34, MyoG, and activation of Notch signaling stimulate in number and activity of Sc modulating and delaying Sp progression; however, detection in biological fluids of GDF8, p16INK4a, Mrf4 and activation of the Wnt pathway would contribute to early Sp development by directly inducing reduced and/or altered Sc function, which would attenuate skeletal muscle restorative capacity. **Conclusions:** The identification of these new biomarkers has the potential to change the way medicine could predict in real-time the course of Sp, by monitoring its evolution and evaluating responses to potential therapeutic strategies.

[1] Dumont NA., et al. (2015). Satellite cells and skeletal muscle regeneration. Compr. Physiol. 5, 1027-1059.
[2] Cho MR., et al. (2022). A Review of Sarcopenia Pathophysiology, Diagnosis, Treatment and Future Direction. J. Korean. Med. Sci. 37, e146.
[3] Fernández-Lázaro D., et al. (2020). Liquid biopsy as novel tool in precision medicine: Origins, properties, identification, and clinical perspective of cancer's biomarkers. Diagnostics. 10, 215.

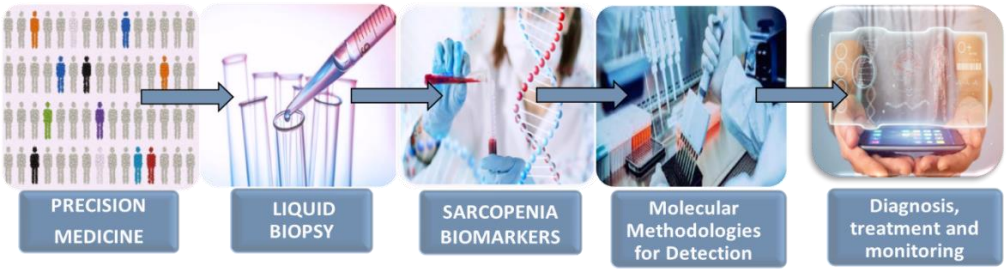


Figure 1. *The operational flow of liquid biopsy to capture the biological and/or functional dynamics of sarcopenia by biomarker analysis.*

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Three-dimensional reconstruction of the primary cilium from ultrathin serial sections

Baselga M.[1], Junquera C.[1,2] and Iruzubieta P.[2]

[1] Instituto de Investigación Sanitaria Aragón (IIS Aragón), Zaragoza, Spain; [2] Department of Anatomy and Histology, University of Zaragoza, Zaragoza, Spain

Introduction: Transmission electron microscopy (TEM) allows high resolution two-dimensional images; however, it lacks information about the three-dimensional disposition of the sample. Although there is technology available to obtain volumes of micrometric structures, these do not have sufficient resolution for ultrastructural analysis (e.g. micro-computerized tomography) or require specialized equipment (e.g. electron cryotomography). We present a simple technique for obtaining ultrastructural volumes using TEM of ultrathin serial sections.
Method: Samples have been processed following the routine electron microscopy protocol. Once the semi-thin sections have been made to locate the area, serial ultra-thin sections between 30 and 60 nm are made, collecting an average of 14 sections on each grid that will be observed under the electron microscope following the predetermined order. Once micrographs of the region of interest have been obtained, the Fiji – Image J software [1] will be used for three-dimensional reconstruction. The basic process consists of three phases: alignment, calibration, and 3D reconstruction. The first phase consists of alignment, where the images of each section are located under the same reference points. In the second phase, calibration is carried out to determine the depth between layers. Finally, in the third phase, the structures of interest are segmented, and the geometry is obtained as a 3D model.

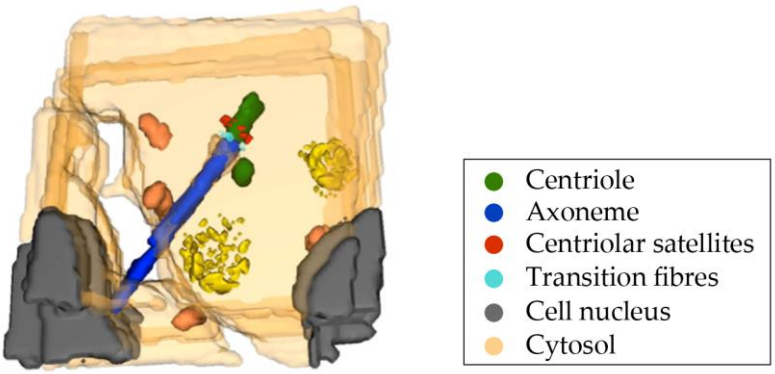


Fig 1. Primary cilium 3D reconstruction.

Discussion & conclusions: The 3D reconstruction of cellular and subcellular components allows a better understanding of the ultrastructure. This technique makes possible to observe all the organelles and cellular structures instead of an isolated section of the sample. It makes possible to complete the interpretation of the conventional ultrastructural analysis. Furthermore, for less experienced researchers or those from other fields of biomedicine, 3D reconstruction facilitates the interpretation and visualization of cellular elements. In this communication, we share a simple method to reconstruct in 3D the subcellular structures observed by TEM ultrathin serial slices.

Authors would like to acknowledge the use of Servicio General de Apoyo a la Investigación (SAI), Universidad de Zaragoza and to Mario Soriano from Centro de Investigación Príncipe Felipe, Valencia, Spain.

[1] Schindelin, J.; Arganda-Carreras, I.; Frise, E.; Kaynig, V.; Longair, M.; Pietzsch, T.; Preibisch, S.; Rueden, C.; Saalfeld, S.; Schmid, B.; et al. Fiji: An Open-Source Platform for Biological-Image Analysis. *Nat. Methods* **2012**, *9*, 676–682, doi:10.1038/nmeth.2019.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Histochemical identification of proteoglycans in chemically-fixed tissues

Sánchez-Porras D.[1,2], Varas J.[3], San Martín S.[3], Godoy-Guzmán C.[4], Bermejo-Casares F.[1], Ortiz-Arrabal O.[1,2], García J.M.[1,2], Carmona R.[5], Campos A.[1,2] and Carriel V.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Centro de Investigaciones Biomédicas, Escuela de Medicina, Facultad de Medicina, Universidad de Valparaíso, Chile; [4] Centro de Investigación Biomédica y Aplicada (CIBAP), Escuela de Medicina, Universidad de Santiago de Chile, (USACH), Santiago, Chile; [5] Department of Cell Biology, Faculty of Sciences, University of Granada, Granada, Spain. Contact: vcarriel@ugr.es

Introduction: Proteoglycans (PGs) are extracellular matrix (ECM) molecules composed of a protein core to which a variety of glycosaminoglycans are anchored. PGs are essential for the biomechanical and functional properties of tissues due to their ability to bind water molecules. PGs may interact with cells, other ECM molecules, and soluble molecules and growth factors [1]. PGs are often involved in numerous pathological processes and play key roles in tissue regeneration processes, and thus their histological evaluation could be needed. There are several histological techniques available to identify PGs, but they offer different degrees of specificity and sensitivity. For this reason, the aim of the present work was to determine the specificity and sensibility of different PGs staining methods in tissues with different amounts of these molecules.
Methods: In this study, we used rabbit tissue samples -ear elastic cartilage (EC) and knee articular cartilage (AC)- and human tissue samples -skin (S), uterine tube (UT), and full-term placenta (PL)-. All these tissues were fixed in 4% neutral buffered formaldehyde, processed, and embedded in paraffin. Sections of 5-µm thickness were stained with hematoxylin-eosin (HE) to study the morphological pattern of the tissues. Then, three histochemical techniques were used to identify tissue PGs: alcian blue pH 2.5 (AB), toluidine blue (TB), and safranin O (SO).
Results: The techniques used here showed different sensitivity when detecting PGs. The technique with the highest sensitivity was AB, which was able to detect small amounts of proteoglycans in tissues with a lower proportion of these components, such as S and UT. In tissues with higher contents of these components, such as EC, AC, and PL, the AB, TB, and SO, all techniques were able to detect PGs, but with certain differences among samples. In the case of EC, the AB technique preferentially reacted with the PGs allocated at the pericellular matrix of the chondrocytes (territory), whereas SO and TB stained all the cartilage ECM. Regarding the AC, more sensibility was obtained with TB, which stained all AC layers, including the calcified cartilage. Interestingly, AB and SO reactions were similar, and more restricted to the area between the superficial and the radial zones. In addition, the TB metachromatic reaction occurred in function of the PGs concentration, as expected.
Discussion & conclusions: The histological techniques evaluated in the present work demonstrated their usefulness in the identification of PGs in animal and human tissues. The AB technique was shown to be the most suitable for routine use due to its high sensitivity, but low specificity. For proteoglycan-rich tissues, such as the human and rabbit cartilage, the three methods demonstrated to be effective, but SO and TB techniques showed higher sensitivity than AB. These results highlight the importance to choose the appropriate histological technique for each case, taking into account the target tissue features and its histological properties, as well as the amount of PGs and the resources available for the study.

Supported by the Spanish Plan Nacional de Investigación Cientifica, Desarrollo e Innovación Tecnológica (I+D+i) of the Spanish Ministry of Science and Innovation, grant FIS PI20/0318, Instituto de Salud Carlos III. Supported by Plan Andaluz de Investigación, Desarrollo e Innovación (PAIDI 2020), Consejería de Transformación Económica, Industria, Conocimiento y Universidades, Junta de Andalucía, Spain, grants P18-RT-5059 and A-CTS-498-UGR18 (proyectos de I+D+i en el marco del Programa Operativo FEDER Andalucía 2014-2020, University of Granada). Co-financed by the European Regional Development Fund (FEDER - ERDF).

[1] Sánchez-Porras D., et al. (2022) Histochemistry of single molecules (book). 2ed Series Methods in Molecular Biology, Springer

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Furin and pcsk4 comparative tissue-level expression-pattern analysis in wild type and infertile mice

Velado-Eguskiza A.[1], Ranero del Olmo A.[1], Gómez-García I.[1], Gómez-Santos L.[1], Crende O.[2], Sáez F.J.[1] and Alonso E.[2*]

[1] Department of Cell Biology and Histology, School of Medicine and Nursing, University of the Basque Country (UPV/EHU), Spain; [2] Department of Cell Biology and Histology, Faculty of Pharmacy, University of the Basque Country (UPV/EHU), Spain. **corresponding author:* edurne.alonso@ehu.eus

The GOPC protein (Golgi-associated PDZ and coiled-coil motif-containing protein) participates in acrosome formation during spermiogenesis. Its knockout shows a phenotype of globozoospermia and subsequent infertility, since the participation of GOPC in the formation of the mature spermatozoon is vital for its success. The study of this anomaly could help in the process of identifying and solving the current worldwide fertility problem and/or aid in the development of non-hormonal male contraceptive methods. The proprotein convertase family plays a key role in post-translational modifications of proteins in the secretory pathway of cells, and is subsequently responsible for the regulation of many processes both inside and outside the cell. In a previous communication to this congress we shared qPCR results showing that the RNA-level expression of three of these convertases (furin, pcsk4 and pcsk6) was significantly decreased in globozoospermic mice compared to wild type, and we described the histological expression pattern of one of them: pcsk6. Much remained to be explored, including the expression patterns of both the ubiquitous furin and the testis-specific, acrosome-related pcsk4. In this work we set out to uncover the tissue-level expression patterns of furin and pcsk4, and to compare each with its globozoospermic counterpart and the other two convertases.

To analyze the expression and distribution of furin and pcsk4 at the tissue level, wild-type C57 BL/6 mice and gopc-/- globozoospermic mice were used. To determine the exact localization of the target proteins, classical immunohistochemical protocols were used in which the visible signal was revealed with DAB.

Starting with the furin expression pattern, it can be seen that it is more intense in the interstitial areas between the seminiferous tubules than inside them, where a clear signal is observed inside the primary spermatocytes and in the head of the elongated spermatids. This signal is mostly maintained when looking at the testes of gopc-/- mice, although the cell morphology itself is affected. As for pcsk4, the results observed are similar to what the literature reports: there is soft staining in primary spermatocytes, which migrates as spermatogenesis progresses towards the proacrosomal vesicle in round spermatids. In gopc-/- globozoospermic individuals, however, the protein studied is not detected in the spermatids, but there is staining in the primary spermatocytes. This fact may be related to the disruption of acrosomal transport in these individuals lacking acrosome. In this work we analyze the variation in the patterns of localization and distribution of these three convertases in globozoospermic individuals with absence of normal acrosomal development.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Effect of temperature on the histological organization of testis and epididymis and on the presence of spermatic maduration indicators in the lizard *Lepidophyma gaigeae*

Uriostegui-Escoto D.[1], Méndez-De la Cruz F.[2], García-Lorenzana M.[3] and Arenas-Ríos E.[3]

[1] Master in Biology of Animal Reproduction, Metropolitan Autonomous University, México; [2] Department of Zoology. National Autonomous University of Mexico, México; [3] Department of Reproductive Biology. Metropolitan Autonomous University, México.

Abstract: The main factor that interferes in practically all the functions of an ectothermic organism is temperature, influencing its formation, growth and development. In addition, exercising optimal temperature control is important for different metabolic and physiological functions, which influence their behavior and reproduction. In this last aspect, directly influencing the correct sperm synthesis, differentiation and maturation [1]. Thermoregulation depends largely on the environment in which the organism finds itself and develops in order to achieve optimal performance [2]. Over time, the environmental temperature has shown a significant increase in recent years, which has had a direct impact on the environment and life of ectothermic organisms [3]. These results have allowed us to infer that reproduction is one of the aspects affected by the increase in temperature [4], in view of this, it is important to know what is happening with reptiles and if it is important to focus on this point for the understanding and effort for the conservation of these species.

The present study focuses on evaluating the effect of temperature on the histological organization of the testis and epididymis and on the presence of sperm maturation indicators of the lizard *Lepidophyma gaigeae*, in the surroundings of Landa de Matamoros, Querétaro. Individuals collected during the month of December 2020 were examined to evaluate the viability, concentration, DNA integrity, presence of the cytoplasmic droplet (CD) in relation to the increase in environmental temperature, as well as the tissue organization of the seminiferous epithelia, epididymis and of the testicular and epididymal interstitial space, as well as to determine the changes in the proportion of proteins with phosphorylation in tyrosine residues, by means of two treatments: at average preferred temperature (Tpref) and at the lower limit of it (Tb), as well as a control group. Sperm parameters revealed a notable decrease in viability in individuals subjected to Tpref, as well as in its concentration at the testicular and epididymal levels. Likewise, the CD evaluation showed a retention at the cauda level, while the Tb and Control groups showed only 5% in this terminal area of the epididymis. The percentage of spermatozoa with intact DNA was also decreased in the Tpref group compared to the Tb and Control groups. Regarding the testicular and epididymal histological organization, the partial results show the identification of typical cell types in the tissues and areas, without apparent cell damage, but with a decrease in the number of sperm in the groups subjected to Tpref. In addition, the finding of apparent extracellular vesicles in the epididymal samples of the Control and Tpref groups could show, in the first instance, a type of response mechanism to sperm damage. These momentary results allow us to broaden the panorama regarding how the thermal factor can affect some reproductive parameters in reptiles, reinforcing the repercussions that this present in the medium or long term in these organisms at an ecological level, as well as the creation of preventive strategies to reduce the environmental damage that these results can cause.

[1] Uriostegui-Escoto D. (2021). Efecto de la temperatura en la calidad espermática de la lagartija Sceloporus megalepidurus. Tesis de Maestría. Universidad Autónoma Metropolitana. 2021.

[2] Arenas-Moreno D., Santos R., Muñoz-Nolasco F., Charruau P. and Méndez-de la Cruz F. (2018). Thermal ecology and activity patterns of six species of tropical night lizards (Squamata: Xantusiidae: Lepidophyma) from Mexico. Journal of Thermal Biology. 75, 97-105.

[3] Sinervo B., Mendez-de-la-Cruz F., Miles D., Heulin B., Bastiaans E., Villagran-Santa Cruz M., Lara-Resendiz R., Martinez-Mendez N., Calderon-Espinosa M. and Meza-Lazaro R. (2010). Erosion of Lizard Diversity by Climate Change and Altered Thermal Niches. Science 328:(5980), 894–899.

[4] Quintero R. (2021). Are physiological and reproductive attributes in Sceloporus aeneus (Reptilia: Squamata) influenced by different temperatures? Tesis de Maestría, Universidad Nacional Autónoma de México, Instituto de Biología, Ciudad de México, México, 2021.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Sertoli cells desquamation in the seminiferous tubule during testicular regression due to the exposure to short photoperiod in the Syrian hamster (*Mesocricetus auratus*)

Seco-Rovira V., Beltrán-Frutos E., Martínez-Hernández J., Ferrer C., Serrano-Sánchez M.I., Madrid J.F. and Pastor L.M.

Department of Cell Biology and Histology. School of Medicine, IMIB-Arrixaca. Regional Campus of International Excellence. Campus Mare Nostrum. University of Murcia. (30120).

Introduction: Testicular regression due to the exposure to a short photoperiod in the Syrian hamster (*Mesocricetus auratus*) causes both a loss of testicular volume and a shortening of the seminiferous tubule. This is produced mainly by the massive loss of germ cells and Sertoli cells resulting from an increase in apoptosis. In other seasonal breeding species, the germinal cell loss, mainly due to the loss of round spermatids through desquamation, has been observed. In diverse pathological situations, apoptosis of Sertoli cells and their detached of the basal membrane take place but the sloughing of Sertoli cells in seminiferous tubules lumen is a rare phenomenon. In this communication a desquamative vimentin-positive cells have been found in the seminiferous tubules lumen and the rete testis of photo-regressed Syrian hamster testis due to exposure to short photoperiod.
Material and Methods: For this study, 20 hamsters were used (15 treated with an 8:16 L-D photoperiod for 12 weeks and 5 treated with an 12:12 L-D photoperiod used as controls). Testes were methacarn fixed, paraffin embedded and stained with hematoxylin-eosin. Three testicular regression groups were established (Mild Regression (MR), Strong Regression (SR) and Total Regression (TR)). Once established, the immunohistochemical technique for vimentin as a specific marker to Sertoli cells for both fluorescence and bright field microscope were performed. For the bright field microscope technique, the TUNEL technique used for the identification of apoptotic cells was previously done. For each animal, four random testicular sections were studied and a total of 50 tubular sections were analyzed.
Results: Vimentin-positive cells with characteristic of Sertoli cells were found with regularity in the lumen of seminiferous tubule and rete testis in TR group. Only sporadically vimentin positive cells were found in SR group not being usually TUNEL-positive.
Conclusions: This initial study shows that Sertoli cell desquamation occurs at the end of the testicular regression process. This could be explained either it is an additional factor in the maintenance of the epithelium when it is already completely regressed or because it is a process of elimination of surplus Sertoli cells prior to testicular recrudescence. Funded 19892/GERM/15; Fundación Seneca; CARM.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

The transitional distal segment of seminiferous tubule, tubuli recti and rete testis in Syrian hamster testes: a histochemical study

Martínez-Hernández J., Seco-Rovira V., Beltrán-Frutos E., Serrano-Sánchez M.I., Madrid J.F., Ferrer C. and Pastor L.M.

Department of Cell Biology and Histology. School of Medicine, IMIB-Arrixaca. Regional Campus of International Excellence. Campus Mare Nostrum. University of Murcia. (30120).

Introduction: Intratesticular ducts in the hamster were studied many years ago using light microscopy. Three portions were recognized: a transitional distal segment (TDS) of the seminiferous tubules; the tubuli recti; and the rete testis. Recently, the TDS portion has brought the attention of researchers because it presents possible Sertoli cell progenitors. In the present communication a histological and immunohistochemical study of both the epithelial portion and the interstitium in these locations was performed.
Material and Methods: For this, a total of 6 hamsters were subjected to a 16:8 light-dark photoperiod. Parasagittal slices of testicular mediastinum region were Bouin or formaldehyde 4% solution fixed and paraffin embedded. The routine Hematoxylin & Eosin and the red picrosirius staining for collagen were performed. Immunohistochemistry for various cell cycle proteins, intermediate filaments and myofilaments and cytokeratins was performed using an avidin-biotin peroxidase method.
Results: Histological observations determined the presence of a new zone between TDS and tubuli recti with a plane epithelium. In the rete testis the epithelium was or flat or cubic simple. The tubular wall showed two layers of myoid cells from TDS to the rete testis. The collagen in the interstitium was more abundant than between seminiferous tubules in these locations. The interstitium around these zones showed abundant Leydig cells being, some of them, positive to c-Kit. Groups of Leydig cells were found in the tunica albuginea and in the initial portion of the rete testis. Immunohistochemical observations showed, in the TDS, positive Sertoli cells for Vimentin, Desmin, Gata 4, Actin and Cyclin D1. In the tubuli recti, epithelial cells were positive for Cytokeratin 8 while this cells between tubuli recti and TDS displayed an intermediate phenotype, with cells similar to Sertoli cells or like the tubuli recti epithelial cells. In the rete testis, epithelial cells were positive for Desmin and Actin. Besides, in this zone as well as in tunica albuginea myofibroblasts in the basal connective tissue probably were positive for actin.
Conclusions: A) In the TDS, Sertoli cells are a heterogeneous population with cells in different states of differentiation. B) A new zone is described between the TDS and tubuli recti portions with epithelial cells composed by Sertoli-type cells and epithelial cells similar to those found in the tubuli recti. C) Leydig cells show a higher area density in the interstitium around the TDS and tubuli recti than between the seminiferous tubules. D) A population of Leydig cells is also observed in the initial portion of the rete testis. Some Leydig cells show characteristics of cells undergoing differentiation.
Funded by GERM 19892/15 from Fundación Seneca CARM.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Vimentin expression in Leydig cells in normal and cryptorchid pig testes

Beltrán-Frutos E., Seco-Rovira V., Serrano-Sánchez M.I., Martínez-Hernández J., Ferrer C., Madrid J.F. and Pastor L.M.

Department of Cell Biology and Histology. School of Medicine, IMIB-Arrixaca. Regional Campus of International Excellence. Campus Mare Nostrum. University of Murcia. (30120).

Introduction: Cryptorchidism is a pathology that inhibits the process of testicular descent in one or both testis during or after birth. This alteration can cause sterility and even trigger tumors. The species that most suffer from this anomaly are humans and pigs, the latter being used as a model to understand cryptorchidism in humans. Among the tissue alterations suffered by the testis, those that occur in the testicular interstitium have been the least studied, with steroidogenesis being one of the affected functions. Therefore, the objective of this work is to carry out a histological and histochemical study of the porcine testicular interstitium, focusing on the modifications suffered by Leydig cells in cryptorchidism.
Material and Methods: For this, we have carried out the study in 3 healthy postpubertal boars, 3 with unilateral spontaneous cryptorchidism and 3 affected with bilateral spontaneous cryptorchidism. We have performed the Picrosirius Red histochemical technique for the study of collagen and the immunohistochemical technique for vimentin as a marker related to steroidogenesis.
Results: The histology of the testicular interstitium differs mainly between healthy and cryptorchid testes, with no significant morphological differences in Leydig cell population between normal and scrotal testes of pigs with unilateral cryptorchidism. Most of the Leydig cells of the scrotal testes presented cytoplasmic positivity to vimentin. This was more intense at the edges of the cell or in the vicinity of the nucleus. Leydig cell positivity to vimentin decreased considerably in abdominal testes with an increase of pyknotic Leydig cells non-vimentin-positive was observed. In these testes there was also an increase in collagen both in the interstitium and in the lamina propria of the seminiferous tubules.
Conclusions: Vimentin, considered important for the steroidogenesis of Leydig cells - since it intervenes in the movement of cholesterol to the mitochondria- has made it possible to differentiate the populations of Leydig cells studied. In fact, the decrease in its positivity in the Leydig cells of the testes with bilateral cryptorchidism correlates with the decrease in the steroidogenic capacity of the Leydig cells in this pathology. Likewise, no changes in vimentin immunoreactivity are seen in the Leydig cells of the scrotal testis of unilateral cryptorchidism, and the immunostaining changes in the abdominal testis of unilateral cryptorchidism are not as strong as those found in cells of Leydig of the testes with bilateral cryptorchidism. Funded by GERM 19892/15 from Fundación Seneca CARM.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Ovarian tissue viability after vitrification and culture

Chávez-Genaro R.[1], Bonjour L.[1], Anesetti G.[1], Barrera N.[2] and Kimelman D.[2]

[1] Biology of Reproduction Group, Department of Histology and Embryology, Universidad de la República, Montevideo, Uruguay,[2] Centro de Esterilidad, Montevideo, Uruguay

Introduction: Ovarian cortex cryopreservation is an acceptable fertility preservation technique and is no longer considered experimental [1] for patients who must undergo oncological treatments. Moreover, ovarian tissue banking is the only method to preserve fertility for prepubertal girls since ovarian stimulation and IVF are not options [2]. However, despite being increasingly applied, ovarian tissue cryopreservation is practiced differently by different groups worldwide [3] In the laboratory of reproductive biology at our institution, we have started fine-tuning the technique using an ovine model. In this work we show the evaluation of morphological parameters of ovarian tissue after vitrification and culture.
Methods: Ovine ovaries were collected from San Jacinto slaughterhouse. Each ovary was cut into half. One half of each ovary was fixed immediately in 4% paraformaldehyde; the other halves of each ovary was introduced into a saline solution and transported at 4°C or room temperature. Once in the laboratory, the ovaries were processed to obtain the cortex of each ovary by dissection or using a manual device that allowed obtaining small pieces of ovarian cortex. Ovarian cortex sections were vitrified using a vitrification protocol [4]. After the vitrification process ovarian cortex was stored in liquid nitrogen for 2 hours approximately and then warmed and cultured for 4 or 20 h. Tissue obtained after each step was fixed, embedded in paraffin, cut to 5 µm, clipped into 5 um and stained with Hematoxylin and Eosin (H-E) to be evaluated for structural changes of tissue sections. The expression of cleaved-CASP3, PCNA and FOXO3a were analyzed by immunohistochemistry
Results: Transport temperature does not cause changes in the tissue architecture. Primordial follicles and oocytes measures were 23.9±1.4 µm and 20.6±1 µm respectively, distributed in a strip of tissue located at approximately 205.40 ± 35.7 µm from the surface. Histological evaluation with H-E does not reveal a significative increase in the percentage of follicles damaged by the vitrification and warming process, however the percentage of atretic follicles increases with time of culture. No significant changes were observed in the analyzed parameters associated with the tissue processing. Quantification of apoptosis and cell proliferation by immunohistochemistry has been difficult, since the number of primordial follicles per section was variable, even in some of them the presence of primordial follicles was not evident.
Discussion & conclusions: Our data shows that further research and analysis on ovarian tissue is needed in order to standardize our vitrification technique. It is also necessary to elaborate techniques that will allow an adequate selection of the tissue to be vitrified.

Supported by ANII ALI 120191154053.

[1] Practice Committee of American Society for Reproductive Medicine. Fertil Steril. 2019;112(6):1022-1033
[2] Silber S, et.al. Fertil Steril. 2010;94:2191–6
[3] Cashmore DA et.al. Reprod Sci. 2021;28(8):2129-35
[4] Laronda MM, et.al. J Asst Reprod Gen.2017;34(2):291-300

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Histology Core Facility at CABIMER: Helping research for 12 years

Rodríguez-Martínez D.[1,2], Remesal-Gordon P.[1,2] and Rojas A.[1,3]

[1] Andalusian Molecular Biology and Regenerative Medicine Centre (CABIMER), Seville, Spain; [2] Fundación Progreso y Salud Consejería de Salud y Familia, Seville, Spain; [3] Universidad Pablo de Olavide, Seville, Spain

Histology, as a branch of the morphological sciences, is a very relevant discipline that allows to understand the shape and structure of tissues, and the characterization of abnormalities at the cellular level.

CABIMER has established a very specialized histology service in order to respond the needs of the researches, including tumor tissue characterization, embryo histology, and animal pathology. The samples collected for analysis are treated with the highest quality standards and with the latest technology, providing a full range of histology services to our research community, as well as the neighbor academic and private sectors.

The Histology Core Facility was created in May 2010 as an internal service and since then the demand from different users has greatly increased. In last years, we have extended our techniques to different species, including invertebrates, becoming an important support for other academic and research institutions.

The histology laboratory offers advice, protocols and equipment allowing fixation techniques, sectioning of tissues and classical and specific stainings for easy viewing of samples. Specific protocols will be provided on demand and upon availability.

The facility offers methods for the histological analysis of human and animal biological samples. Some of the available methods in this service include the preparation of paraffin embedded samples in the automatic processor of tissue, which simplifies the work of the researches regarding the manipulation of samples and duration of the protocol. For paraffin blocks and frozen tissues, histological sections are generated using an automatic microtome and cryostat, respectively. The unit also provided floating samples using a vibratome.

The Histology Core Facility also perform intracardiac perfusion of mice. This process includes from the manipulation of the alive animal to the generation of the histological sections of interest.

The Histology Core Facility has established methods for performing histological studies on samples from cell cultures, such as cell pellets, and from organoids. For this, we use a special gel called Histolgel™, which allows us to prepare paraffin embedded special samples in the automatic tissue processor.

Other technique that The Histology Core Facility offers is the generation of Tissue Microarrays (TMAs), where representation of animal or human tissues (up to X number of samples) can be analyzed at the same time by immunohistochemistry or immunofluorescence.

The facility is also equipped with a Cytospin for the processing of biological fluids and cell cultures. The unit is also responsible for new users training and advice in the available equipment. Advanced users have free access to the core facility under internal online booking.

Supported by Consejería de Transformación Económica, Industria, Conocimiento y Universidades de la Junta de Andalucía-Fondo: Fondo Social Europeo, Iniciativa de Empleo Juvenil - Nº Expediente POEJ_00027

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Expression of basement membrane-related molecules by Wharton Jelly stromal cells in the full-term human umbilical cord

Sánchez-Porras D.[1,2], Bermejo-Casares F.[1,2], Varas J.[3], Campos F.[1,2], Pérez-Sabio J.[4], Fahim-Koury E.[5], Pozzobon M.[6], San Martín S.[3], Carmona R.[7] and Carriel V.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Centro de Investigaciones Biomédicas, Escuela de Medicina, Universidad de Valparaíso, Reñaca, Chile; [4] Servicio de Obstetricia y Ginecología, Hospital Virgen de las Nieves, Granada, España; [5] Servicio de Neurofisiología Clínica, Hospital Universitario San Cecilio, Granada, España; [6] Stem Cells and Regenerative Medicine Lab, Fondazione Istituto di Ricerca Pediatrica Città della Speranza & Department of Women's and Children's Health, University of Padova, Padua, Italy. [7] Department of Cell Biology, Faculty of Sciences, University of Granada, Granada, Spain. Contact: davidsp@ugr.es; vcarriel@ugr.es

Introduction: Stem cells are orthotypical undifferentiated cells that have a high turnover rate and retain the ability to differentiate towards cells of different lineages [1]. Maintenance of these capabilities is directly related to the microenvironment surrounding these cells, the so-called cell niche [2]. Some of these niches, such as the basal stratum of epithelia, are highly influenced by the presence of a basement membrane that creates a barrier between the cells and the subjacent tissue. The umbilical cord, in particular the Wharton's jelly tissue, is a source of mesenchymal stem cells (WJSC) widely used in tissue engineering. WJSCs were used to generate 3D microtissues *in vitro*, and they surprisingly produced a basal membrane-like structures with collagen IV *ex vivo* [1]. However, the expression of basal membrane molecules in human umbilical cords is poorly known. This study aims to determine which basal membrane components form part of the native WJSCs niche, which could contribute to better understand the potential of WJSCs in tissue engineering.
Methods: Full-term human umbilical cords were obtained, fixed in 4% formalin or methacarn and embedded in paraffin. 5 µm-thick sections were subjected to histochemical and immunohistochemical techniques to analyze the structure of this tissue and the expression of relevant molecules in the basal membrane, inclucing type IV collagen, laminin, perlecan and agrin. In addition, samples were fixed in 2.5% glutaraldehyde and embedded in epoxy resin for transmission electron microscopy (TEM) analysis.
Results: Histological analysis with HE showed a scattered cell distribution pattern, with no apparent order in the Wharton's jelly region, as expected. Histochemistry conducted with Alcian blue revealed the presence of a high amount of proteoglycans, while picrosirius red staining clearly stained the collagen content. PAS showed a slight positivity around some WJSCs, as well as the presence of glycogen granules. Interestingly, immunohistochemistry confirmed the expression of collagen type IV, laminin, perlecan and agrin basal membrane molecules with a clear pericellular pattern around the WJSCs. Furthermore, TEM confirmed these findings, suggesting the presence of a thin basal membrane-like pericellular matrix around these cells.
Conclusions: The extracellular matrix of the Wharton's jelly tissue showed a homogeneous distribution of proteoglycans allocated within a collagen-rich extracellular matrix, which could contribute to retain high amounts of water in this mucous tissue. This histological study demonstrated that WJSCs have a highly complex pericellular matrix surrounding each cell, with positive expression of collagen type IV, laminin, perlecan and agrin. These results suggest the presence of an incipient basement membrane-like structure around these cells. The role of this structure is unknown, but it could be important to maintain the WJSCs niche in the umbilical cord. Although the potential role of these molecules should be determined in future studies, the presence of these molecules opens the door to the future use of WSJCs in tissue engineering of complex tissues requiring the establishment of a basement membrane, such as the human cornea limbus.

Supported by grant CSyF PI-0086-2020 from the Consejería de Salud y Familias, Junta de Andalucía, Spain, and grant B-CTS-504-UGR20 (proyectos de I+D+i en el marco del Programa Operativo FEDER Andalucía 2014-2020, University of Granada and Consejería de Transformación Económica, Industria, Conocimiento y Universidades). Cofinanced by the European Regional Development Fund (ERDF) through the “Una manera de hacer Europa” program.

[1] Durand-Herrera D et al. Histochem Cell Biol. 2018;150(4):379-93
[2] Saggioro M, D'Agostino S. et al. (2021). Biomater Sci. 2021 Dec 21;10(1):124-137

SESSION

HISTOLOGY OF ORGANS, APPARATUSES AND SYSTEMS

HISTOLOGÍA DE ÓRGANOS, APARATOS Y SISTEMAS

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Ciliogenesis in normal and neoplastic Thyroid C cells

Vázquez-Román V.[1], Sánchez López J.[1], Fernández-Santos J.M.[1], and Martín-Lacave I[1].

[1] Department of Normal and Pathological Cytology and Histology, School of Medicine, University of Seville, Spain.

Background: Thyroid gland is an endocrine organ composed by two endocrine cell types. The most abundant population are follicular cells which produce thyroid hormones and the minor population are the -calcitonin producing- C-cells (CC). The presence of primary cilia (PC) in endocrine cells such as pancreatic islets, adenohypophysis and adrenal cortex cells is widely known [1]. With regard to the thyroid gland, in humans, follicular cells project at least one PC towards the follicular lumen, however, in rats, PC are unidentifiable in the postnatal life [2]. The presence of PC in mammals CC, however, is currently unknown. Considering that PC has been related to the functional pathology of the thyroid gland [3], the objective of this study is: to clarify whether CC possess PC in both human and rat thyroids and, in that case, to analyse the differences between normal and neoplastic CC.
Methods: Consecutive tissue sections from: human thyroid glands, (2 normal thyroid (NT) and 4 medullary thyroid carcinomas (MTC), and from 6 WAG/Rij rat CC carcinomas (CCC) were analysed. First, sections were stained with Hematoxylin-Eosin (H-E) for pathologic diagnosis and then samples were immunostained for calcitonin (CT) to label CC. In order to find the method that would adequately mark both PC and CC, different antibodies were tested by double immunofluorescence (IF).
Results: The presence of PC was demonstrated by double IF in both human and rat normal CC. However, in tumours, depending on the combination of antibodies used, some limitations were found. The combination of α-Tubulin and CT prevented the specific identification of PCs due to the high signal from the tubulin present in the cytoplasm of neoplastic CC [4]. In addition, the intense labelling of CC with CT also masked PC. For this reason, we tried to label CC using E-Cadherin. However, since E-Cadherin is frequently lost along the development of malignant epithelial tumours [5] this marker was not suitable either (Fig 1). Finally, we used ARL13b and CT, and we were able to adequately stain both the PC and CC (Fig 2). PC in CC maintained their elongated shape although they appeared to be shorter than the PC in thyrocytes. In neoplastic samples, their frequency decreased as compared to normal CC and also some interspecies differences were observed, being scarce and irregular in the CMT and more frequent and regular in CCC (Fig 3).
Conclusions: - PC are present in CC in both human and rat thyroids. The differences observed between normal and neoplastic CC open possible pathways for the treatment of these tumours being PC a potential new therapeutic target.

Supported by CTS- 439/2017 (Diffuse Neuroendocrine System).

[1] Iwanaga T., et al, (2011). Restricted expression of somatostatin receptor 3 to primary cilia in the pancreatic islets and adenohypophysis of mice. Biomed. Res 32(3),225-35.
[2] Utrilla JC., et al. (2015) Comparative study of the primary cilia in thyrocytes of adult mammals. J. Anat. Oct 227(4),550–60.
[3] Fernández-Santos JM., et al (2019) Primary Cilium in the Human Thyrocyte: Changes in Frequency and Length in Relation to the Functional Pathology of the Thyroid Gland. Thyroid. Apr 1;29(4):595–606.
[4] Zabel M. and Grzeszkowiak J. (1997) Histology and Histopathology From Cell Biology to Tissue Engineering Characterisation of thyroid medullary carcinoma TT cell line. Histol Histopathol 12:283–9.
[5] Steiner F., et al (2016) Expression of tenascin C, EGFR, E-Cadherin, and TTF-1 in medullary thyroid carcinoma and the correlation with RET mutation status. Int. J. Mol. Sci Jul 1,17(7).

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

SEM and ATR-FTIR study of teeth exposed to tobacco, alcohol and/or medication

Garrosa E.[1], Díez C.[2] Rojo M.A.[3], Martín-Gil J.[4], Martín-Ramos P.[5], Fernández-Lázaro D.[6], Garrosa M.[1] and Córdoba-Díaz D.[7]

[1]Department of Cell Biology, Histology and Pharmacology, and INCYL, University of Valladolid, Valladolid, Spain; [2]Health Sciences, Miguel de Cervantes European University, Valladolid, Spain; [3]Area of Experimental Sciences, Miguel de Cervantes European University, Valladolid, Spain; [4]Agriculture and Forestry Engineering Department, ETSIAA, University of Valladolid, Palencia, Spain; [5]University Institute of Research in Environmental Sciences of Aragon (IUCA), EPS, University of Zaragoza, Huesca, Spain; [6]Department of Cell Biology, Histology and Pharmacology, Faculty of Health Sciences, Campus of Soria, University of Valladolid, Soria, Spain; [7]Department of Pharmaceutics and Food Technology, and IUFI, Complutense University of Madrid, Madrid, Spain.

Teeth are continuously exposed to oral environment, being influenced by diet, medication, stress, mineralization agents and individual and other factors. Teeth are subject to demineralization/remineralization cycles due to the action of saliva, in which the pH is determinant, conditioning tooth mineral composition and therefore its hardness, with consequences in the response to dental curative and preventive treatments. In the present study we aimed to analyze the changes occurred in teeth surface and mineral composition of enamel and cementum of patients showing smoking habit, alcohol consumption and/or prescription of anti-inflammatory or psychotherapeutic drugs. We employed scanning electronmicroscopy (SEM), energy-dispersive X-ray spectroscopy (EDS) and attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR). 36 permanent molars and incisors from patients aged between 21 and 78 years old were obtained from a dental clinic and divided in 5 groups: smokers, alcohol consumers, taking anti-inflammatory drugs, taking psychotherapeutic drugs, and control. Friedman's nonparametric test was used for statistical analysis.

SEM study revealed well preserved structure of enamel and cementum, enamel showing aprismatic areas and perikymata. Qualitative differences in the stratification process between enamel and cementum could be seen. No distinct structural differences were found across the different groups. The regions adjacent to plough furrows, made for obtaining the powder for analysis, evidenced the arrangement pattern of hydroxyapatite crystallites. Elemental analysis by EDS revealed that smokers showed the lowest Ca/P ratio. Enamel crystallinity was highest in control patients and increased with patient's age as well as with the amount of tobacco inhaled. On the contrary, cementum crystallinity decreased with patient's age. The degree of mineralization was higher in enamel than in cementum, smokers taking a psychotherapeutic drug showing highest mineralization. In the root, type A carbonate underwent the greatest alteration, especially in patients under analgesic and psychotherapeutic treatment, and then in elderly patients consuming alcohol. The amide band I (-CO-NH_2), representative of protein secondary structure, showed similar profile for all analyzed samples, being relatively higher in young smokers and smokers under treatment with anti-inflammatory medication.

In summary, ATR-FTIR holds promise in dentistry research providing quantification of calcium and phosphate concentration in hydroxyapatite as well as its crystallinity, and therefore allowing for the determination of the degree of mineralization of enamel and cementum. The remineralization process appears more active in enamel than in cementum due to direct contact with saliva. Smoking habit and some psychotherapeutic drugs negatively affect calcium balance in tooth surface, albeit no obvious change was observed on this surface with SEM, what suggest that remineralization process develops rapidly and homogeneously from its initial form due to the rapid flow of Ca^{++} and PO_4^{3-} ions occupying the space left as water and mineral salts are leaving. Ca/P ratio changes varies as a function of the mineralization degree, thus it may be used as an index of remineralization.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Anti-tumoral modulation of liver microenvironment by selective depletion of reactive hepatic stellate cells

Arteta B.[1], Cano S.[1], Gragera I.[1], Sansuán L.A.[1] and Benedicto A.[1]

[1] Department of Cell Biology and Histology, School of Medicine and Nursing, University of the Basque Country, UPV/EHU, Leioa, Spain

Introduction: Hepatic stellate cells (HSCs) are non-parenchymal liver resident cells with a wide array of functions. They remain in a quiescent state in the space of Disse, in close contact with hepatocytes and liver sinusoidal endothelial cells. However, upon activation acquire a myofibroblast-like phenotype, showing contractile ability. Activated HSCs play a major role during tissue repairing, extracellular matrix regulation, immune response and inflammation. Even though HSCs are major actors in liver fibrosis, their involvement in the generation and regulation of the hepatic tumor microenvironment needs further attention.

Material and methods: We used a previously described transgenic (TG) mice expressing the herpes simplex virus thymidine kinase gene (HSV-Tk) driven by the mouse GFAP promoter, based on the differential expression of GFAP in the liver cells, mostly constrained to HSCs. Therefore, the limited expression of HSV-Tk on proliferating GFAP-expressing HSCs makes them susceptible to apoptotic cell death upon Ganciclovir (GCV) metabolization. To analyze in vivo the effect of active HSCs depletion in the tumor hepatic microenvironment, we injected intra-splenically either murine B16-F10 melanoma or murine MC38 colon carcinoma cells, and allowed them to generate liver metastasis in wild type (WT) and TG mice with or without GCV treatment, and metastatic developments was analyzed. The modulation of pro-tumoral parameters was analyzed by immunohistochemistry. Moreover, we analyzed *ex vivo* the cytotoxic potential against tumor cells of intra-sinusoidal lymphocytes isolated from tumor-bearing mice. Furthermore, we examined the activity of Mannose Receptor on liver sinusoidal endothelial cells (LSECs) isolated from tumor-bearing mice, and the cytotoxic potential of intra-sinusoidal lymphocyte after co-culture with the mentioned primary LSECs.

Results: In vivo, the treatment of MC38 or B16 tumor bearing mice with GCV substantially reduced the foci number and total area occupied by liver metastasis around 80 %. Moreover, livers from TG mice showed reduced key tumor microenvironment parameters, such as intratumoral collagen accumulation, neoangiogenesis and recruitment of inflammatory cells in the liver, accompanied by modulation of LSECs-ManR activity. This was also related to changes in the cytotoxicity of lymphocytes towards tumor cells, either after isolation from hepatic sinusoids or after co-culture with primary LSECs isolated from tumor-bearing mice.

Conclusion: HSCs depletion resulted in a decrease in the metastatic progression accompanied by the modulation of key features of the liver pro-metastatic microenvironment, such as angiogenic, desmoplasic, and inflammatory responses. Our results point out HSCs as a required spark for the progression of liver metastasis, making them a good candidate for new therapies to treat liver metastasis of different primary origins by targeting the tumor microenvironment.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Histomorphometric analysis of human palatal mucosa with emphasis on connective tissue grafts

García-Caballero L.[1], Gándara M.[1,2], Cepeda-Emiliani A.[1], García-Caballero T.[1,2], Gallego R.[1], Gude F.[3], Suárez-Quintanilla J.[4] and Blanco-Carrión J.[5]

[1] Department of Morphological Sciences (Histology Area), School of Medicine and Dentistry of Santiago de Compostela University and Health Research Institute of Santiago (IDIS), Santiago de Compostela, Spain; [2] Department of Pathology, University Clinical Hospital, Santiago de Compostela, Spain; [3] Epidemiology Unit, University Clinical Hospital and Health Research Institute of Santiago (IDIS), Santiago de Compostela, Spain; [4] Department of Morphological Sciences (Anatomy and Embryology Area), School of Medicine and Dentistry, Santiago de Compostela University, Santiago de Compostela, Spain; [5] Department of Surgery and Medical-Surgical Specialties, School of Medicine and Dentistry, University of Santiago de Compostela, Santiago de Compostela, Spain

Introduction: Gingival recession is defined as the oral exposure of the root surface caused by displacement of the gingival margin apical to the cement-enamel junction [1] and is highly prevalent worldwide [2]. To solve this problem, connective tissue grafts emerged as treatments that could provide significant improvements. To carry out soft tissue replacement grafts it is essential to know the histology of the donor site. The aim of this study was to analyze the histomorphometric characteristics of hard palatal lining and its different areas to assess which is the donor site of choice for connective tissue replacement grafts.

Methods: Six human cadaver heads (all male, aged >70 years), donated to the Faculty of Medicine for educational and research purpose, were used. Palatal samples including the entire thickness of the palatal lining were obtained. From each palate, samples were harvested at four different sites: (1) incisal, (2) premolar, (3) molar and (4) tuberosity, as well as an anteroposterior strip for a general view. Samples were stained with hematoxylin eosin technique. From each section, the thickness of the epithelium (EP), lamina propria (LP) and submucosa (SM) were measured (PathScan) at three different locations: coronal, medial and apical to the gingival margin. Results were expressed as mean ± SD. Comparison of thickness of the different palatal regions were performed by analysis of variance (ANOVA). Tukey's multiple comparison test was used to determine which set of means differ from the rest. Differences were considered statistically significant at $p<0.05$.

Results: All samples were suitable for image analysis and then 24 sections were analyzed. The mean thickness of the palatal mucosa was 4.42±1.72 mm. Analyzing the different layers separately, the mean thickness of the EP was 0.24±0.08 mm (6.45% of the total thickness), that of the LP was 1.34±1.54 mm (34.31%) and that of the SM was 2.84±2.31 mm (59.25%). The comparative study of the thickness of the different tissue layers showed no significant differences for the EP in the four regions analyzed. In the LP, significant differences were observed ($p<0.001$), with similar values in the first three regions, and a significantly greater thickness in the tuberosity (3.64±1.46 mm) ($p<0.001$). The thickness of the SM was also different between the regions studied ($p<0.001$), increasing from incisal to molar, and without evidence of this layer in the tuberosity. Excluding the EP, the means of thickness percentage of LP and SM were 37% and 63%, respectively ($p<0.001$). The percentage of the thickness occupied by the LP decreases from incisal (24.52%) to premolar (15.42%) and molar (8.05%), reaching the maximum in the tuberosity (100%), and being the differences significant ($p<0.001$).

Discussion and Conclusions: Excluding the epithelium, the percentage of the thickness of lamina propria and submucosa in our samples was 37% (slightly more than 1/3) and 63% (slightly less than 2/3), respectively. Similar results were obtained by other authors [3]. In our samples, no submucosa was found in tuberosity, although other authors reported a small percentage of submucosa in this location (4.9%) [4]. In conclusion, as LP is the tissue of choice for volume augmentation in autologous grafts, based on our results the best donor sites would be firstly the tuberosity, followed by the incisal site, due to the high amount of LP in these areas, being SM scarce or absent.

[1] Chambrone LA, Chambrone L. J Periodontol. 2006; 77(5):909-916.
[2] Merijohn GK. Periodontol 2000. 2016; 71(1):228-242.
[3] Marques de Mattos P, et al. J Periodontal Implant Sci. 2020; 50(1):2-13.
[4] Sanz-Martín I et al. Clin Oral Investig. 2019; 23:957-964.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Ovarian effects of vincristine-based chemotherapy and their eventual attenuation by dichloracetate

Toledo A.[1], Hernández K.[1], Anesetti G.[1] and Chávez-Genaro R.[1]

[1] Histology and Embryology Department, Facultad de Medicina, Universidad de la República, Montevideo-Uruguay

Introduction: Female reproductive competence is determined by her ability to produce quality mature oocytes. The follicular structures in which oocytes develop are highly susceptible to substances with gonadotoxic effect, including many of the chemotherapy drugs, causing alterations in fertility. Vincristine, used for the treatment of hematological diseases, acts by disrupting microtubules, arresting cell proliferation. However, its effects on the ovary are poorly studied [1]. Recent works shows that vincristine induce mitochondrial damage. In the oocyte, mitochondria support numerous processes during maturation, fertilization and early embryonic development, so they are closely related to oocyte quality and ovarian aging. On the other hand, Dichloroacetate (DCA) is a modulator of mitochondrial activity. This drug favors oxidative over glycolytic metabolism, which would improve the quality of the oocyte and the survival of the ovarian follicles [2]. This evidence allows us to hypothesize that therapies optimizing mitochondrial activity could result in improved oocyte survival against vincristine-induced toxicity.

Methods: An adult-young murine model was used, including animals exposed for 2 weeks to: 1) vincristine; 2) DCA in drinking water; 3) vincristine concomitantly with DCA and 4) controls that received water and vehicle. At the end of treatment, the histoarchitectural characteristics of the ovary and follicular population, were evaluated. The patterns of cell proliferation and death were characterized by immunohistochemical analysis.

Results: Groups exposed to vincristine showed a decrease in the percentage of healthy preantral follicles compared to the unexposed groups; the presence of DCA did not induce changes in this parameter. The percentage of antral follicles was similar in any of the treatments. Activated anti-caspase 3 immunohistochemistry revealed a significant increase in the percentage of antral atretic follicles in the vincristine-exposed groups with respect to control ($p \leq 0.05$), and significant differences between both groups exposed to DCA ($p \leq 0.05$). The group exposed to vincristine also presented a lower proportion of primordial follicles with nuclear-localized anti-FOXO3a immunoreactivity than that observed in the control group ($p \leq 0.05$). Treatment with DCA showed no significant differences.

Discussion & conclusions: Our results show that vincristine induces an increase in apoptosis in the pool of growing follicles, and an increase in the proportion of primordial follicles that are being activated. The administration of DCA does not seem to reverse the processes of atresia of the growing follicles with the parameters so far analyzed.

Supported by Comisión Sectorial de Investigación Científica (CSIC). UdelaR

[1] Winship.A, et al. Toxicol Sci. 2019; 169(1):43–53
[2] Miquel, E. et al, PLoS ONE. 2012; 7(4):1–9.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Possible direct release of material synthesized in the rough endoplasmic reticulum by mesenchymal cells in the extracellular matrix of human amniotic membrane

Cortes S. [1], Seco-Rovira V. [1], Beltrán-Frutos E. [1], Blanquer M. [2], Ferrer C. [1], Martínez-Hernández J. [1], Serrano-Sánchez M.I.[1] and Pastor L.M. [1]

[1] Department of Cell Biology and Histology, School of Medicine, IMIB-Arrixaca, University of Murcia. Regional Campus of International Excellence. Campus Mare Nostrum. University of Murcia.30120. [2]Cell Therapy Unit. Haematology Department. University Hospital Virgen de la Arrixaca (HUVA). IMIB-Arrixaca.

Introduction: The amniotic membrane (AM) mesenchymal cells are responsible for synthesizing the extracellular matrix of the compact and spongy layers of this tissue. Our research group has found that there is a degree of heterogeneity in these mesenchymal cells related to a higher fibroblastic differentiation in mesenchymal cells with a high positivity to fibronectin and vimentin in their cytoplasm. Ultrastructurally, these cells are classified into various types by their increased dilated rough endoplasmic reticulum and heterochromatin in the nucleus. In more differentiated cells, the synthetized material is accumulated in the cytoplasm due to the membrane fusion of the endoplasmic reticulum. The objective of this communication is to investigate whether or not, there is some type of mechanism for this material release into the extracellular matrix that surrounds these cells.

Material and Methods: AM of 4 placentas at 39 weeks of gestation were used. AM extracted from periumbilical zone were fixed and processed for transmission electron microscopy studies. For this, samples (1 mm^3) were fixed for 4 h in 3.5% glutaraldehyde in 0.1 M sodium cacodylate buffer. They were then washed in 0.855 mg/100 ml saccharose-cacodylate, postfixed in 1% osmium tetroxide (for 2 h), dehydrated in a graded acetone series and embedded in Epon 812 (Serva). Semithin sections were stained with toluidine blue. Ultrathin sections were cut using a Reichert-Imy® Ultracut Ultramicrotome, contrasted with uranyl acetate and lead citrate, and examined with a Zeiss® EM/10CR electron microscope.

Results: Four types (I, II, III, IV) of mesenchymal cell were characterized ultrastructurally as in previously studies. The types III and IV showed dilated cisternae of rough endoplasmic reticulum with a material similar to the surrounded extracellular matrix. In some occasions this material was denser and acquire a globular shape. In diverse cells the membranes of reticulum were very close to plasmatic membranes and occasionally a communication among lumen endoplasmic reticulum and extracellular matrix was observed. Moreover, globular material was found around of mesenchymal cells in the extracellular matrix probably derived of themselves.

Conclusions: A) the material synthetized in rough endoplasmic reticulum with high amount of fibronectin is release to extracellular matrix in a direct form. B) It is possible that this release was related to the senescence condition of mesenchymal cells. C) The deposits released could participate in the alteration and rupture of fetal membrane in the labour. Funded by GERM 19892/15 from Fundación Seneca CARM.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

New tissue classification and its implications in the field of Tissue Engineering and Regenerative Medicine

Garcia-Montero C.[1,2,*], Fraile-Martinez O.[1,2], García-Honduvilla N.[1,2], Ortega M.A.[1,2] and Bujan J.[1,2]

[1] Universidad de Alcalá, Department of Medicine and Medical Specialties, Faculty of Medicine and Health Sciences, 28801 Alcalá de Henares, Spain; [2] Ramón y Cajal Institute for Health Research (IRYCIS), 28034 Madrid, Spain; * Corresponding author: cielo.gmontero@gmail.com

The concept of tissue is the fundamental pillar of the development of Histology and Tissue Engineering. Traditionally, four large groups of tissues have been distinguished: epithelial tissue, connective tissue, muscle tissue and nervous tissue. This classification was based on the study of the structure and composition of the different organs of the body, recognizing these large functional groups. Once again, technological advances are the tools that allow us to provide new insights into the cells of the different tissue groups in the body. In this line, some research within the ENCODE project (Encyclopedia of DNA Elements) according to extensive transcriptional profiles analyzed from 53 primary human cells obtained from different locations. have redefined five types of histological tissues (epithelial tissue, nervous tissue, mesenchymal tissue, blood tissue and endothelial tissue). Within each identified tissue, there are some singularities with respect to the traditional classification. Although in this new classification we can again find a great embryological correlation. Thus, the epithelial tissue encompasses the epithelial and nervous tissues, the mesenchymal tissue encompasses, as in Bichat's classification, the conjunctive, adipose, cartilaginous, bone, but now the muscular tissue is included and, on the contrary, the endothelial tissue is separated into two independent tissues. and the blood tissue that if we remember have their extraembryonic origin in the yolk sac. Thus, according to the gene expression, these 5 cell groups could be identified, being an identifying signature of the tissues and a reflection of the morphological and histological heterogeneity of the tissues. In addition, the heterogeneity within the same tissue stands out, we can differentiate cellular subpopulations with different gene expression and properties according to its location. Deviations in the composition of the cellular composition of the different tissues are related to histopathological phenotypes. In this communication, it is intended to convey the most important ideas of this new classification of tissues, as well as one of the implications in knowledge of Histology and the applications that may derive from this new classification for Tissue Engineering and Regenerative Medicine.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

In situ characterization of Human Dental Pulp Stem Cells (hDPSC). A histological study

Garzón I.[1,2], Enrique-Cruz Y.[1], González-Gallardo C.[2,3], Ortiz-Arrabal O.[1,2], Chato-Astrain J.[1,2], Martín-Piedra M.A.[1,2] Sánchez-Quevedo M.C.[1,2], Crespo P.V.[1,2], García J.M.[1,2] and Alaminos M.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Division of Ophthalmology, University Hospital Virgen de las Nieves, Granada, Spain

Introduction: Human Dental Pulp Stem Cells (hDPSC) represent a potential stem cell source for use in tissue engineering due to their self-renewal capability, multilineage differentiation potential and immunomodulatory properties [1]. Histologically, the human dental pulp consists of four topographical zones, from the dentin-pulp junction towards the center of the pulp: 1) odontoblastic zone, 2) oligocellular layer of Weil, 3) cell-rich zone and 4) pulp core zone. The zone in which hDPSC reside is not well known, although it has been proposed that the cell-rich layer zone or the pulp core zone could contain most of these cells in the human tooth [2]. In addition, recent studies demonstrated that cells allocated at specific zones of the dental pulp express different markers and express different proteins. The present work is aimed to analyze the histological features of hDPSC corresponding to each specific zone of the human dental pulp in order to determine the potential application of each type of cell in tissue engineering of the human skin, cornea, oral mucosa and palate.

Methods: We analyzed the expression of the undifferentiation markers CD90, CD105 and CD73 and the control marker CD45 in cells allocated *in situ* in the four zones of the dental pulp. In addition, a histochemical analysis was performed to identify the presence of extracellular matrix (ECM) components, including fibrillar and non-fibrillar components using Picrosirius red, Verhoeff, Gomori's reticulin and alcian blue staining methods.

Results: Our results demonstrated that hDPSC have intrinsic capability to express *in situ* all major stem cells markers such as CD90, CD105, CD73 and were negative for CD45, especially in the layer of Weil, the cell-rich zone and the pulp core zone. Interestingly, the expression of CD105 was mainly associated to cells in contact with the blood vessels of the pulp core zone. Regarding the ECM, our semiquantitative analysis showed high amounts of type I collagen in the cell-rich zone and pulp core zone, with scarce number of elastic fibers in the cell-rich zone. The reticular fibers were only identified in the layer of Weil. Finally, the non-fibrillar content of the pulp tissue was specially identified in the cell-rich and pulp core zones.

Discussion & conclusions: Our results suggest that the *niche* of hDPSC is not restricted to the cell-rich layer and the pulp core zone, and was also found in the layer of Weil. In addition, the amount of fibrillar and non-fibrillar components of the ECM of dental pulp tissue tended to vary within the four different zones. This fact could be related to the differences in the cell populations found in each zone and confirms the idea that the environment could control the phenotype of each type of cells and their regenerative potential. Although these results suggest that MSC allocated at the layer of Weil, the cell-rich layer and the pulp core zone could have regenerative properties and could be used in tissue engineering of the human skin, cornea, oral mucosa and palate. However, future studies should confirm the future clinical applications of these cells.

Supported by the Spanish Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I) of the Spanish Ministry of Science and Innovation (Instituto de Salud Carlos III), grants FIS PI18/0331, FIS PI21/0980, FIS PI20/0317, ICI19/00024 (BIOCLEFT) and ICI21/00010 (NANOULCOR), and by grants PE-0395-2019 and PI-0442-2019 from the Consejería de Salud y Familias, Junta de Andalucía, Spain. Addition support was provided through grant B-CTS-450-UGR20 (proyectos de I+D+i en el marco del Programa Operativo FEDER Andalucía 2014-2020, University of Granada and Consejería de Transformación Económica, Industria, Conocimiento y Universidades), and cofinancing was provided from the European Regional Development Fund (ERDF) through the "Una manera de hacer Europa" program.

[1] Tsutsui TW. et al. Stem Cells Cloning. 2020;13:33-42
[2] Verma K. et al. Dent Res J (Isfahan). 2014;11(3):302-8

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Characterization of orthokeratinized and parakeratinized epithelia of human oral mucosa. A histological study

Ibáñez-Cortés M.[1], Chato-Astrain J.[1,2], Ávila-Fernández P.[1], Blanco-Elices C.[1,2], Bermejo-Casares F.[1], de la Cueva-Batanero P.[1], Fernández-Valadés R.[1,2,3], Rodríguez I.A.[1,4], Martín-Piedra M.A.[1,2] and Garzón I.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Division of Pediatric Surgery, University Hospital Virgen de las Nieves, Granada, Spain; [4] Cátedra "B" de Histología y Embriología, Facultad de Odontología, Universidad Nacional de Córdoba, República Argentina

Introduction: The human oral mucosa (HOM) is a complex structure that plays an essential role in protecting the oral cavity. The histological pattern of this structure is tightly related to its function, and important structural differences have been identified among the different anatomical regions of the oral cavity, suggesting an important role of the mechanical forces that are present at each region. Histologically, the HOM can be classified as orthokeratinized, parakeratinized and non-keratinized, with orthokeratinized and parakeratinized corresponding to HOM subjected to mastication forces [1]. Although the histological pattern of the HOM has been extensively studied, there are few studies focused on determining the specific characteristics of each type of keratinized HOM. The present study is focused on determining the main properties of orthokeratinized and parakeratinized HOM.

Methods: Orthokeratinized and parakeratinized HOM samples were fixed in 4.0% w/v neutral buffered formaldehyde, embedded in paraffin, sectioned in 5µm-width sections, and stained with hematoxylin and eosin (H&E) using routine methods. Immunohistochemical methods were then used to detect specific epithelial-related markers, including cytokeratin 13 (CK13), desmoplakin (DSP) and filaggrin (FLG) and the cell proliferation marker Ki67. Finally, the total epithelium thickness and the thickness of each epithelial stratum was quantified in each sample using ImageJ.

Results: The histological analysis revealed differences between orthokeratinized and parakeratinized HOM regarding their epithelial pattern (Figure 1). Although the spinosum stratum was thick in both cases, this layer stratum was more developed in parakeratinized HOM as compared to orthokeratinized HOM. In addition, CK13 expression was detected in suprabasal strata of parakeratinized oral mucosa, but its expression was restricted to the basal and spinosum strata in orthokeratinized samples. FLG expression also varied regarding keratinization degree, and we found a homogenously distributed expression of this marker in the corneum stratum of the orthokeratinized epithelium, and a diffuse and discontinuous expression in the corneum stratum of parakeratinized HOM. Ki67 and DSP showed no remarkable differences regarding the expression pattern between both types of samples.

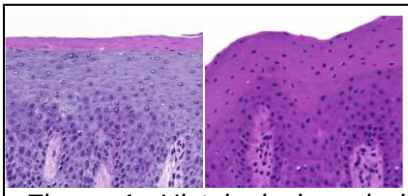


Figure 1. Histological analysis of orthokeratinized (left) and parakeratinized (right) HOM stained with H&E.

Discussion & conclusions: Our preliminary results suggest that the orthokeratinized and parakeratinized HOM share important structural and molecular similarities. However, some differences exist at the epithelial level, which could be related to the different biomechanical forces associated to each type of HOM. These findings could contribute to a better knowledge of the human oral mucosa histology and could be useful for the future generation of bioartificial substitutes of each type of human oral mucosa by tissue engineering techniques [2].

Supported by the Spanish Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I) of the Spanish Ministry of Science and Innovation (Instituto de Salud Carlos III), grants FIS PI18/0331, FIS PI21/0980, and ICI19/00024 (BIOCLEFT), and by grant PI-0442-2019 from the Consejería de Salud y Familias, Junta de Andalucía, Spain. Cofinancing was provided from the European Regional Development Fund (ERDF) through the "Una manera de hacer Europa" program.

[1] Madani, et al. 2014:98(6):1225–1238
[2] Sánchez-Quevedo, et al. Histology and histopathology. 2007:22(6):631–640

SESSION

HISTOPATHOLOGY

HISTOPATOLOGÍA

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Determination of HER2 and Microsatellite Instability in Gastric Cancer. Clinicopathological Implications

Bermúdez A.[1], Arranz-Salas I.[2,3], Ortega M.V.[2,3], Mercado S.[2], López-Villodres J.A.[2], Sánchez-Varo R.[2], Escamilla A.[4], Rodríguez-Pérez L.M.[2], Alba C.[2], Hierro I.[3] and Bermúdez D.[2]

[1]Department of Anesthesiology, Nuestra Señora de Valme University Hospital, 41014 Seville, Spain; [2]Department of Human Physiology, Human Histology, Anatomical Pathology and Physical Education. University of Malaga, 29010 Malaga, Spain;[3] Unit of Anatomical Pathology, Virgen de la Victoria University Hospital, 29010 Malaga, Spain;[4] Unit of Radiation Oncology, Virgen de la Victoria University Hospital, 29010 Malaga, Spain

Introduction. Gastric cancer (GC) is one of the leading causes of cancer-related death. Although different morphological classifications of GC have been proposed, they lack clinical utility, as they have no prognostic or predictive value. GC is a complex, heterogeneous and multifactorial disease, and its molecular characterization could establish different types to enable the individualization of patients; hence, the importance of the new molecular classifications of GC.

The combination of new molecular classifications with clinicopathological parameters would make it possible to distinguish different groups of patients, developing new therapeutic strategies, improving gastrointestinal oncology, and bringing us closer to precision medicine. Our aim was to correlate two molecular types of GC: HER2-positive (human epidermal growth factor receptor 2) and microsatellite instability (MSI) with clinicopathological data to assess whether this could influence treatment or prognosis.

Methods. A retrospective study of 142 GC patients was performed with molecular characterization through HER2 overexpression and DNA repair protein expression for MSI. Immunohistochemistry was used for molecular analysis. The clinicopathological data collected were as follows: age, sex, location, pathological diagnosis, histological type according to the Lauren classification, degree of differentiation, TNM classification and stage, lymphatic, vascular and perineural involvement, perioperative chemotherapy, and survival.

Results. The general clinicopathological features of our GC patients are advanced age, male sex, intestinal type, proximal location and stages II-III. The percentage of HER2-positive tumors was 13.4%, predominantly in men. Correlations were found with intestinal type, presence of metastases, advanced stages and chemotherapy. Almost 75% of HER2-positive patients died. It was associated with a poor prognosis. MSI occurred in 16.2% of the cases, significantly associated with advanced age, female sex, distal location and intestinal type. These patients showed few lymph node and metastases, with a predominance of early tumor stages. The percentage of deaths was lower among MSI patients who did not receive perioperative chemotherapy and were treated with surgery alone. MSI GC was associated with better prognosis.

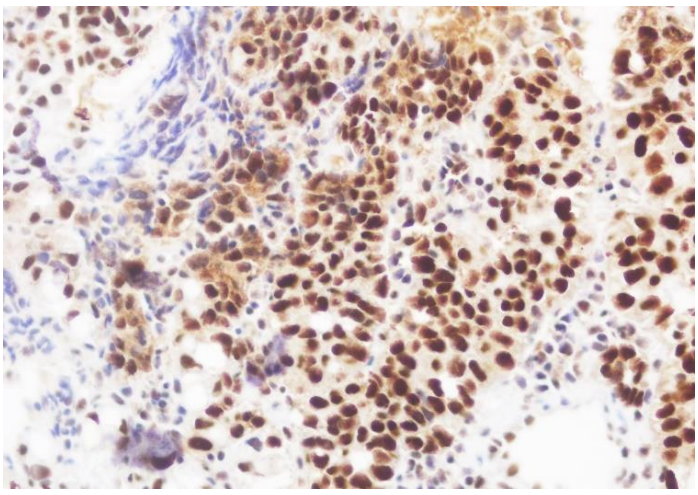


Figure 1. HER2-positive gastric cancer. Immunohistochemical technique showing intense basement membrane and basolateral staining in more than 10% of the cells (400x)

Conclusion. HER2 and MSI status in GC is important for their association with specific clinicopathological features and for their prognostic and predictive value. The data supports the importance of determining these molecular types of GC, considering that they are not always routinely evaluated.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Spot the difference! Basal cell adenoma versus Basal cell adenocarcinoma

Gil-Belmonte M.J.[1,2], Cuello-Entrena E.[1], Cámara-Pérez J.[2,3], Pinochet-Almonacid S.[1] Valdenebro-Cuadrado G.[1] and Sobrino-Prados A.[1]

[1] Department of Pathology, Torrecárdenas University Hospital, Almería. Spain; [2] Department of Morphological Sciences, Section of Histology, Faculty of Medicine and Nursing, University of Córdoba, Spain; [3] Department of Plastic, Esthetic and Reparative Surgery, Reina Sofía University Hospital, Córdoba, Spain.

Background: Basal cell adenoma and basal cell adenocarcinoma are rare salivary gland neoplasms that most commonly arise in the parotid gland between the fifth and sixth decades of life. This work aims to illustrate their differential diagnosis by comparing two cases that demonstrate the preponderant role of Histology in the diagnostic process.
Methods: A 65-year-old man and a 73-year-old woman consulted at the ENT service because of a slow-growing right-sided facial nodule and an adherent left-sided retroauricular mass. Superficial and partial parotidectomy were respectively performed after imaging confirmation of exact anatomic location. Samples were collected and sent to the Pathology service for their analysis.
Results: Grossly, specimens could be distinguished by the presence/absence of a defined capsule and the homogeneity/heterogeneity of the cut surface. Histological examination revealed a double population of a cord, nested architectural pattern in the first case, and a mixed nested and anastomosing trabecular pattern with a hyaline matrix in the second one. Necrosis, dystrophic calcifications, and a higher mitotic rate (Ki67 20-80%) were also observed at the latter, showing high-grade transformation and linfovascular invasion. CKAE1/AE3 and CK7 stained positive for epithelial and myoepithelial cells in both tumors; p63 demonstrated strong diffuse positivity at the basal cells of the adenoma. Immunoreactivity for BER-EP4 was evidenced at the basaloid component of the adenocarcinoma.
Discussion & Conclusions: Basal cell adenoma and basal cell adenocarcinoma characteristically show biphasic basaloid and epithelial cellularity. Basal cell adenocarcinoma differs from its benign analogous in its infiltrative and invasive growth pattern as well as in the presence of mitosis and necrosis. Although ancillary techniques might serve as help in the differential diagnosis of these tumors, morphological assessment on routine hematoxylin-eosin is still key for an adequate classification and, thus, for better patient management.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Non-invasive cancer detection as a precision medicine tool: liquid biopsy of androgen receptor variant 7 (AR-V7) in prostate cancer

Fernández-Lázaro D.[1,2], Garrosa E.[3] and Garrosa M.[2,3]

[1]Department of Cell Biology, Histology and Pharmacology, Faculty of Health Sciences, University of Valladolid, Campus de Soria, Spain; [2]Research Group in Neurobiology, Faculty of Medicine, University of Valladolid, Spain; [3]Department of Cell Biology, Histology and Pharmacology, and INCYL, University of Valladolid, Valladolid, Spain

Background: Androgen receptor variant 7 (AR-V7), contributes to rapid progression, more aggressive, and higher recurrence in prostate cancer (PC). Its determination by liquid biopsy allows precision oncology pharmacology to monitor PC heterogeneity, target individualized therapies, and evaluate responses [1]. Enzalutamide, a new-generation androgen receptor antagonist, represents a novel therapeutic solution in metastatic castration-resistant PC (mCRPC) [2]. Therefore, we determined the efficacy of Enzalutamide in 16 mCRPC patients as a function of AR-V7 expression. **Methods:** Assessment of AR-V7, in plasma obtained by Liquid Biopsy, in 16 mRCPC patients using messenger ribonucleic acid (mRNA) proteins by WESTM® (capillary electrophoresis + nanoimmunoassay; ProteinSimple USA). Collection from the clinical history: age at diagnosis, metastatic location, prostate-specific antigen (PSA), Gleason index, and time to progression. **Results:** AR-V7+ (n=8) vs AR-V7- (n=8) patients set earlier age at diagnosis of mCRPC (68 vs 79 years); decreased PSA response rate (25 vs 15 ng/ml); increased cancer aggressiveness (Gleason ≥8 vs ≤6); progression-free time decreased 10-fold (2 vs 20 months); and increased metastatic development (8: 3 bone + visceral; 2 nodal + bone; 3 bone vs 3: 1 bone + visceral; 1 nodal + bone; 1 bone). **Discussion:** The level of AR-V7 expression increases significantly during prostate cancer progression and correlates with the risk of tumor recurrence after radical prostatectomy. Moreover, the ability to respond to new hormonal agents, such as Enzalutamide, in mCRPC is conditional on the expression of the AR-V7 biomarker, which is key in the personalized therapeutic selection, providing clinically determinant prognostic information that will advance the patient's health. **Conclusions:** The assessment of the presence of AR-V7 in plasma of PC patients is possible by means of a novel capillary nanoimmunoassay (Figure 1). AR-V7 is more frequent in aggressive tumors, which projects ARV-7 as a new biomarker in cancer potentially detectable in biofluids by liquid biopsy.

[1] García-Hernández JL., et al. (2017). A novel capillary nano-immunoassay for assessing androgen receptor splice variant 7 in plasma. Correlation with CD133 antigen expression in circulating tumor cells. A pilot study in prostate cancer patients. Clin. Transl. Oncol. 19, 1350-1357.
[2] Antonarakis ES., et al. (2014). ARV7 and resistance to enzalutamide and abiraterone in prostate cancer. N. Engl. J. Med. 371, 1028–38.

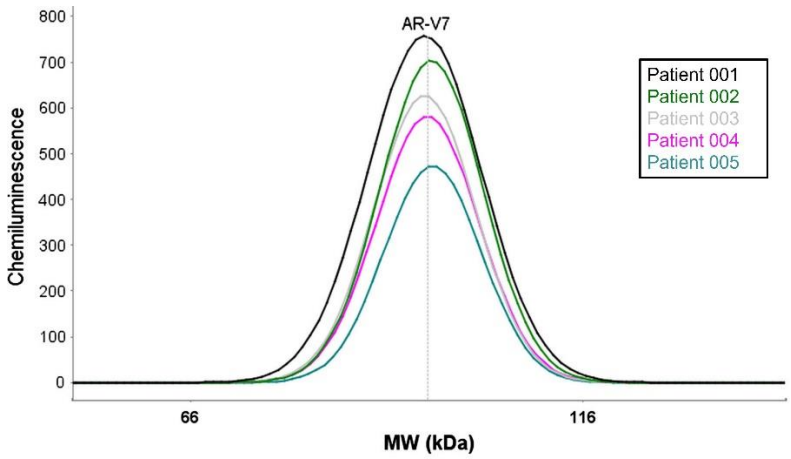


Figure 1. *Nanoimmunoassay by WESTM® using an anti-AR-V7 antibody. Peaks of AR-V7 signal in patients*

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Role of IRS-4 in the origin of multifocal hepatocellular carcinoma: effects of Actinomycin D

Ortega M.A.[1,2*], Fraile-Martinez O.[1,2], Garcia-Montero C.[1,2], Saez M.A.[1,2,3], Monserrat J.[1,2], Alvarez-Mon M.[1,2,4], García-Honduvilla N.[1,2], Bujan J.[1,2] and Guijarro L.G.[2,5]

1Universidad de Alcalá, Department of Medicine and Medical Specialties, Faculty of Medicine and Health Sciences, 28801 Alcalá de Henares, Spain. 2Ramón y Cajal Institute for Health Research (IRYCIS), 28034 Madrid, Spain. 3Department of Pathological Anatomy, Hospital Universitario Central de la Defensa-UAH, 28001 Madrid, Spain. 4Department of Internal Medicine and Diseases of the Immune System-Rheumatology, Oncology (CIBEREHD), Hospital Universitario Príncipe de Asturias, 28806 Alcalá de Henares, Spain. 5Universidad de Alcalá, Unit of Biochemistry and Molecular Biology, Department of Systems Biology (CIBEREHD), 28801 Alcalá de Henares, Spain *Corresponding author: miguel.angel.ortega92@gmail.com

Actinomycin D (ActD) is an FDA-approved NCI oncology drug that specifically targets and downregulates stem cell transcription factors, leading to a reduction of stem cells within the tumor mass. Recently, our research group has shown the importance of IRS-4 in the development of liver cancer. In this study, we evaluated any protective effect of IRS-4 against ActD. For this study, three hepatocellular carcinoma cell lines (HepG2, Huh7, and Chang cells) were used to study the mechanism of actinomycin D. Most assays were performed on the Hep G2 cell line, due to the high expression of biomarkers. of stem cells. We found that ActD causes necroptosis of HepG2 cells characterized by DNA fragmentation, decreased mitochondrial membrane potential, cytochrome c depletion, and decreased reduced glutathione levels. However, we did not observe a clear increase in apoptosis markers such as the presence of annexin V, caspase 3 activation or PARP fragmentation. ActD produced an activation of MAP kinases (ERK, p38 and JNK) and AKT. Activation of AKT and MAP kinases induced by ActD produced an activation of the Rb-E2F cascade together with a blockade of cell cycle transitions, due to c-jun depletion. ActD led to inhibition of pCdK1 and pH3 along with DNA fragmentation, leading to cell cycle arrest and subsequent activation of p53-dependent cell death in the HepG2 cell line. Only JNK and AKT inhibitors protected against the effects of ActD. N-acetyl-L-cysteine also had a protective effect as it restored GSH levels. A likely mechanism for this is that IRS-4 stimulates GCL-GSH and inhibits the Brk-CHK1-p53 pathway. The assessment of IRS-4 in cancer biopsies could be of interest for personalized treatment with ActD.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Histological analysis of unexpected pathological findings in a transgenic animal model of pancreatic cancer

Martínez-Herrero S.[1], Narro-Íñiguez J. [1], Martínez-Moral M.P.[1,2] and Martínez A.[1]

[1] Center for Biomedical Research of La Rioja, Fundación Rioja Salud, Logroño, Spain; [2] University of La Rioja, Logroño, Spain

Pancreatic cancer has a very high mortality rate, being responsible for more than 432,000 annual deaths worldwide [1]. Early detection is essential for the success of any cancer treatment. However, the lack of symptoms and the absence of specific biomarkers in early stages prevent the early detection of this malignancy. Most pancreatic cancers are diagnosed at stage IV, when surgery is not an option, resulting in low survival rates: 24% at one year and only 9% at 5 years [1]. Our main goal is to find new biomarkers for the early diagnosis of pancreatic cancer. Since finding patients in the early stages of the disease is difficult, we carried out a study using an animal model of pancreatic cancer that has been perfectly characterized [2]. In this model, tumors are induced in the mouse pancreas by the endogenous expression of the Kras oncogene under the control of the promoter PDX1-Cre. Two genotypes were compared: cancer [Flox Kras$^{+/-}$; Pdx1-Cre$^{+/-}$] and control [Flox Kras$^{+/-}$; Pdx1-Cre$^{-/-}$]. Experimental animals (n=9/group, males and females) were sacrificed at different ages (1, 2, 2.5, 3, 4 or 6 months) to create a temporal profile of tumor growth. However, unexpected pathological conditions affected the animals on the cancer group, especially at 3 and 4 months. For that reason, different organs (colon, cecum, duodenum, stomach, liver, spleen and thymus) were also measured, collected, and studied.

In the cancer group, 42.5% of the animals exhibited some kind of pathological symptom, the most common being rectal bleeding and skin tumor growths. Only 3.7% of the control animals showed a pathological condition, namely dermatitis. Females were more susceptible to these unexpected conditions (62.5% of the affected animals were females) and, so far, no female has reached the 6 months time point. All animals in the cancer group got at least one more affected organ, other than the pancreas, being the duodenum the most affected (100%), followed by non-malignant skin tumors (21%), colon (18%), thymus (15%), and spleen (12%). Histological analysis of the different organs revealed that, surprisingly, the duodenum affectation was limited to its macroscopical aspect (increased weight), but no inflammatory cells were found under the microscope. In the colon, a significant increase of goblet cells was observed, but tissue architecture was preserved and small infiltration areas were found only in a few samples. Among other malignancies, thymomas were the most common (15%), followed by lung tumors (9%), and stomach cancer (6%). Despite all these unexpected findings, the pancreatic lesions appeared, as expected, around 2.5 months of age, and progressed spontaneously to invasive and metastatic ductal adenocarcinomas over time (Fig.1).

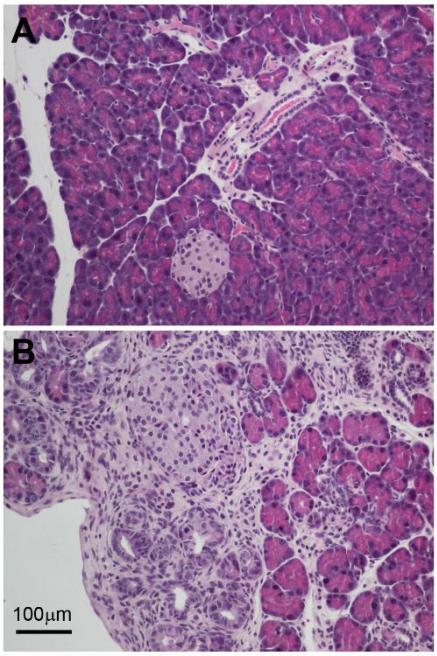


Fig. 1. Representative H&E micrographs of normal pancreas (A) and pancreatic ductal adenocarcinoma (B) in 4-month old mice.

These results suggest that, although this model causes pancreatic cancer that progresses with time, as expected, it also causes a plethora of other pathologies in different organs. Therefore, this should be taken into account when using this transgenic model and different contingency measures should be implemented during the study.

Supported by Roche Farma (Stop Fuga de Cerebros) and Agencia de Desarrollo Económico de La Rioja (ADER, Hequipa project). M.P.M.-M. is the recipient of a Sara Borrell contract.

[1] Rawla P, et al. World J Oncol. 2019; 10(1):10–27
[2] Hingorani SN, et al. Cancer Cel. 2003; 4(6):437-50

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Analysis of the effect of embryonic hyperglycemia on the development of the ventricular myocardium and the expression of molecules marking damage to the myocardium

Jaime-Cruz R.[1,3], García-Lorenzana M.[2], Lazzarini-Lechuga R.[2], Villavicencio-Guzmán L.[3], Salazar-García M.[3] and Sánchez-Gómez C.[3*]

1. Posgrado en Biología Experimental, Universidad Autónoma Metropolitana Iztapalapa, 2. Departamento de Biología de la Reproducción, Universidad Autónoma Metropolitana Iztapalapa, 3. Laboratorio de Biología del Desarrollo y Teratogénesis Experimental, Hospital Infantil de México Federico Gómez.

Introduction. Gestational hyperglycemia (HGG) is associated with increased teratogenesis and adverse effects on embryo-fetal development leading to the onset of heart defects [1]. The exact cause of these disorders is still unclear. miRNAs control gene expression in a post-transcriptional manner. Since their discovery, an increasing number of them have been involved in the pathogenesis of HGG. In clinical and experimental investigations, it was determined that the overexpression of miR-223 is associated with an increase in the expression of TNNI3K (heart-specific kinase that binds to cardiac troponin I (cTnI) involved in abnormal cardiac remodeling processes leading to the development of ventricular hypertrophy and heart failure [2]. At the cardiovascular level, cell junctions are essential elements during cardiac embryogenesis, electrical impulse transmission, synchronization, etc. Under pathological conditions, they participate in the development of congenital heart disease, arrhythmogenesis, and abnormal cardiac remodeling. To evaluate the effect of a hyperglycemic environment on the deregulation of miRNA-223 and its relationship with changes in the development of the ventricular myocardium. To evaluate the cardiac embryonic development under hyperglycemic conditions and its relationship with the development of the ventricular myocardium, changes in the expression of the proteins sarcomeric (troponin I cTnI, desmin and TNNI3K), cell junction proteins and the possible deregulation of miRNA-223. METHODOLOGY. Fertile eggs of *Gallus gallus domesticus* were incubated for 3.5 days to obtain embryos at stage 22HH, when the appearance of ventricular buds ends. Hyperglycemia was induced by administering glucose solution (30 mmol/L) in the chorioallantoic membrane every 24 h, for 10 days of incubation (stage 36HH). Control embryos were treated with saline solution. Blood glucose was recorded periodically. The heart was used for histopathological analysis, expression of miR-223 by RT-qPCR and expression detection with confocal microscopy and western blot of proteins. RESULTS. Histopathological analysis showed a decrease in the thickness of the left (30%) and right (25%) ventricular wall in HG embryos compared to controls. RT-qPCR showed a 40% increase in miR-223 in HG embryos compared to normoglycemic embryos. TNNI3K and cTnI respectively showed 39% and 36% greater fluorescence, it was corroborated by western blot in HG embryos compared to controls, in case of cell junctions there was a decrease in expression by 66%, 41% and 71% for ZO1, B-catenin and N-cadherin respectively. Discussion. The hyperglycemic environment during embryonic development decreased fetal maturation. The heart showed smaller size and thickness of the ventricular walls. The increase in miR-223 triggered the elevation of TNNI3K and cTnI that can induce abnormal cardiac remodeling processes. Conclusions. Our findings correlate with the hypertrophy described in fetuses and newborn children of women with HGG.

[1] ADA. (2022). Standards of Medical Care in Diabetes. American Diabetes Association. USA: Journal of diabetes.
[2] Wang H., Wang, L., Song, L. and Meng, X. (2013). TNNI3K is a novel mediator of myofilament function and phosphorylates cardiac troponin I. Braz. J. Med. Biol., 46, 128-137.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Histological evaluation of skin biopsies from patients affected by frontal fibrosing alopecia

Carmona-Rodríguez M.[1], Bermejo-Casares F.[2], Navarro-Triviño F.[3], García J.M.[2,4], Chato-Astrain J.[2,4], Carmona R.[5], Fernández-Valadés R.[2,4,6], Alaminos M.[2,4], Carriel V.[2,4] and Ruiz-Villaverde R.[3]

[1] Department of Dermatology, Hospital General Universitario de Ciudad Real. España; [2] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [3] Servicio de Dermatología, Hospital Universitario San Cecilio, Granada, Spain; [4] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [5] Department of Cell Biology, Faculty of Sciences, University of Granada, Granada, Spain; [6] Division of Pediatric Surgery, University Hospital Virgen de las Nieves, Granada, Spain; Contact: vcarriel@ugr.es

Introduction: Frontal fibrosing alopecia (FFA) is a primary lymphocytic scarring alopecia, characterized by frontal and temporoparietal hairline recession, leading to a cicatricial band. Its prevalence is increasing worldwide since its first description in 1994 by Kossard. The etiopathogenesis of FFA remains unknown, although hormonal factors, autoimmunity, genetic susceptibility, and some exogen factors are thought to play a role. Several treatments have been used in FFA, although at present there is no validated or approved treatment. The aim of this study was to perform a clinico-histological evaluation of samples from patient affected by FFA.
Methods: In this study, 26 patients with FFA were included and two biopsies were taken from each, one from the clinically unaffected scalp (CTR) and other from the alopecic band. An additional biopsy was taken from the area of clinical progression in 6 patients. Biopsies were processed for histology and sections were stained with hematoxylin-eosin (HE), Alcian blue (AB), and picrosirius red (PS). Histology was evaluated by three independent histologists with experience in dermatopathology. The parameters studied included the severity of the perifollicular (PFI) and perivascular inflammatory infiltrate (PVI), the thickness of dermis and epidermis, the presence of perifollicular fibrosis and the presence of melanophages.
Results: Histology showed that the mean epidermal thickness was lower in the alopecic band compared to the control area (42.04 vs 36.76 µm) and so was the dermal thickness (1459.33 µm vs 1210.04 µm). The presence of follicles and sebaceous glands was considerably reduced (observed in 29% and 25% respectively), while the 91% of FFA affected samples contain piloerector muscle. The PFI and PVI were observed in 95% and 100% of FFA samples respectively. Curiously, 75% and 70% of unaffected biopsies presented PFI and PVI. The definition between the papillary dermis and the reticular dermis was poorer in FFA affected samples. Melanophages were more frequently observed in the alopecic band specimens in which epidermal pigmentation was less evident.
Discussion: FFA presents mainly as a recession of the frontotemporal hairline, leaving behind a cicatricial alopecic band. It is histologically characterized by a lichenoid lymphocytic infiltrate around the upper follicle, as well as concentric perifollicular lamellar fibrosis [1]. The epidermal and dermal atrophy found in our study is in line with previous studies [1]. A recently described clinical feature in this disease is the depression of the frontal veins, which appears to be related to this skin atrophy. It has already been recognized that FFA represents a generalized inflammatory disease that involves clinically unaffected areas supporting our histological findings [2]. The increased presence of melanophages may be related to the decreased melanocyte count in the alopecic band, which clinically presents as hypopigmentation [3]. Further studies will be conducted to determine a correlation between our histological findings with clinical aspects of these patients. Moreover, immunohistochemical analyses are needed to determine the immunophenotype of the inflammatory cells observed in these patients.

This study was supported by grant PE-0395-2019 from Consejería de Salud y Familias, Junta de Andalucía, Spain. Co-financed by the European Regional Development Fund (ERDF).

[1] Saceda-Corralo, D. et. al. (2021) J. Eur. Acad. Dermatol. Venereol. 35, e47–e49
[3] Pindado-Ortega C, et. al. (2020) J Eur Acad Dermatol Venereol. Feb;34(2):e84-e85
[3] Katoulis, A.C.; et. al. (2021) J Eur Acad Dermatol Venereol. May;35(5):e343-e345

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Reduction of Caveolin-1 after myocardial infarction: a histological study in myocardium from human autopsies and a swine model

Ruiz-Saurí A.[1,2], Rios-Navarro C.[2], Cantos-Amores G.[2], Molina-García T.[2], Ortega M.[2], de Dios E.[3], Gavara J.[4], Perez-Sole N.[2], Marcos-Garces V.[2], Diaz A.[5], Chorro F.J.[2,3,6,7] and Bodi V.[2,3,6,7]

[1]Department of Pathology, Faculty of Medicine, Universitat de Valencia, Valencia, Spain; [2] Institute of Health Research-INCLIVA. Valencia, Spain; [3]Centro de Investigación Biomédica en Red (CIBER)-Cardiovascular, Madrid, Spain; [4]Universidad Politécnica de Valencia, Valencia, Spain; [5]Unidad Central de Investigación Biomédica (UCIM), Universitat de Valencia, Valencia, Spain; [6]Department of Medicine, Faculty of Medicine, Universitat de Valencia, Valencia, Spain; [7]Servicio de Cardiología, Hospital Clínico Universitario de Valencia, Valencia, Spain.

Introduction: Caveolae are lipid invaginations of 50 to 100 nm that are present in the membrane of most mammalian cells. They are mainly made up of three proteins: caveolin (Cav)-1, 2, and 3 and are involved in signal transduction, ion channels and lipid and protein homeostasis. In addition, caveolae participate in inflammation, fibrosis, and oxidative stress and is proposed to exert a cardioprotective role. Myocardial infarction (MI) consists on the thrombotic occlusion of a coronary artery which has to be rapidly reperfused to avoid massive cardiomyocyte apoptosis. Since caveolae are implicated in the pathophysiology of several entities and regulate different signalling pathways to promote cardiac protection, exploring their implication in MI context could be of interest. Therefore, in order to elucidate whether caveolae are implicated in the pathophysiology of MI, we aim to compare the expression of Cav-1 in the human myocardium isolated from patients with a previous MI and in controls as well as in an experimental model of reperfused MI,

Materials and methods: 1) Myocardial samples from human autopsies of 4 controls and 4 MI patients with more than 6 months of evolution were isolated. 2) MI was induced in swine by means of 90-min of occlusion of the mid left anterior descending coronary artery using angioplasty balloons followed by one-week (n=3) or one-month (n=3) reperfusion. Indeed a control group (n=3) was also included. In both models, the extension of Cav-1 was determined by immunohistochemistry and morphometrically quantified using the image analyzer Image-Pro Plus. Data were statistically compared using unpaired t-Student's test. Statistical significance was considered for a two-tailed p-value<0.05.

Results: A constitutive presence of Cav-1 was observed in the control myocardium from humans, concretely in cardiac muscle and in endothelial cells. When comparing the expression between the peri-infarct region of MI patients and controls, a significant reduction in the expression of Cav-1 was detected by immunochemistry. Lastly, Cav-1 was more expressed in cardiomyocytes than in endothelial cells, but unlike muscle cells, its amount was not diminished after MI. Regarding the data obtained in the experimental model, similar results were obtained: Cav-1 was present in cardiomyocytes and endothelial cells in control myocardium. In comparison to control animals, the extension of Cav-1 was diminished in the cardiac muscle cells from the peri-infarct region in both MI groups. On the contrary, no differences in the expression of Cav-1 in endothelial cells were noted between the three experimental groups.

Conclusion: The presence of Cav-1 is shown to decrease after the MI in both clinical and experimental samples. Further studies are needed to confirm the role of caveolae post-MI and their use as potential therapeutic target.

Fundings: This work was supported by grants from "Instituto de Salud Carlos III" and "Fondos Europeos de Desarrollo Regional FEDER" (grant numbers PI20/00637 and CIBERCV16/11/00486) and by Conselleria de Educación – Generalitat Valenciana (PROMETEO/2021/008).

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Sex-dependent mechanisms of delayed cell death after perinatal asphyxia

Chillida M.[1], Hilario E.[1], Álvarez A.[1], Robertson N.J.[2,3] and Alonso-Alconada D.[1,2]

[1] Department of Cell Biology and Histology, School of Medicine and Nursing, University of the Basque Country (UPV/EHU), Leioa, Bizkaia, Spain; [2] Institute for Women's Health, University College London, London, UK; [3] Edinburgh Neuroscience, Centre for Clinical Brain Sciences, the University of Edinburgh, Edinburgh, UK

Introduction: Perinatal insults like hypoxia-ischemia may produce neuronal cell death and brain damage, leading to neurological disabilities or even the death of the newborn. Males have shown more vulnerability to neonatal brain damage after perinatal asphyxia [1]. Further, sex dimorphism is gaining importance as the response to hypothermia (the only clinical therapy against hypoxia-ischemia) seems to be better in females than in males, and this may be due to the activation of different cell death mechanisms [2]. As the piglet model of neonatal brain injury is closer to the human neonate, we wanted to evaluate i) if there is a sex dimorphism on total cell death after hypoxia-ischemia; ii) the pattern of cell death in males and females; and iii) a possible modulation in the delayed cell death pathways depending on sex.

Methods: 15 piglets (9 females and 6 males) were subjected to a controlled cerebral hypoxic-ischemic insult [3]. After 48 hours, the brain was perfusion-fixed and, at the level of the optic chiasm, 5-µm serial slices were obtained and stained with H&E or immunostained. We quantified total cell death, necrotic, apoptotic, cleaved caspase-3 and cleaved PARP-1 positive cells in five brain regions (cingulate and sensorimotor cortex, periventricular white matter, caudate nucleus and thalamus). Total averaged values and differences between males and females were analysed using Student's t-test or Mann-Whitney. Interregional values were analysed by ANOVA or Kruskal-Wallis. For each test, $p<0.05$ was considered significant.

Results: Male piglets showed higher values of total ($p<0.01$) and necrotic ($p<0.0001$) cell death, whereas females had more apoptosis ($p<0.0001$). The delayed cell death mechanism differed between sexes: female cell death relied on caspase-3 activation while active PARP-1 was prominent in males (Fig.1).

The inter-regional analysis revealed a significant increase in apoptotic cell death and cleaved caspase-3 activation in the cingulate cortex and periventricular white matter in female piglets. For their part, males showed more necrosis in the sensorimotor cortex and periventricular white matter, and cleaved PARP-1 peaked in caudate nucleus and thalamus.

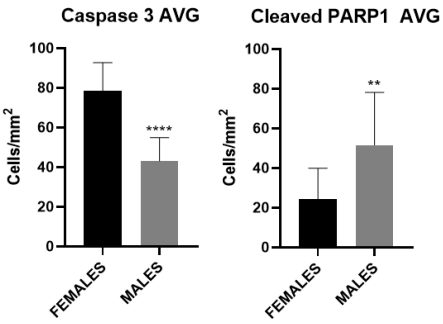


Fig. 1. Average and 95%CI values of cleaved caspase-3 and cleaved PARP-1 positive cells in female and male piglet brains. P<0.01 (**), P<0.0001 (****).

Discussion & Conclusions: These results suggest that the pattern of cell death may be sexually dimorphic in neonatal piglets exposed to perinatal asphyxia, a clinical model more similar to humans than rodents. Future research is needed taking into account the influence of sex together with the specific brain region affected to develop more individualized and precise neuroprotective therapies.

Supported by EITB Maratoia-BIOEF (BIO18/IC/003), and the Spanish Ministry of Science and Innovation (MINECOR20/P66/AEI/10.13039/501100011033).

[1] Raz S., Debastos A. K., Newman J. B., and Batton D. (2010). Extreme prematurity and neuropsychological outcome in the preschool years. J. Int. Neuropsychol. Soc. 16, 169-179.
[2] Wood T. R., et al. (2020). Variability and sex-dependence of hypothermic neuroprotection in a rat model of neonatal hypoxic–ischaemic brain injury: a single laboratory meta-analysis. Sci. Rep. 10, 1-13.
[3] Alonso-Alconada D., et al. (2015). Brain cell death is reduced with cooling by 3.5°C to 5°C but increased with cooling by 8.5°C in a piglet asphyxia model. Stroke 46, 275-278.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Effect of *Aristotelia chilensis* extract in a Crohn disease chronic model

Merinero M.[1,2], Macías-García L.[1], Vázquez V.[1], García-García M.D.[1,3], Ortiz T.[1], García-Esteban I.[1], García-Montes J.M.[4], Alcudia A.[2], Argüelles-Arias F.[3,4] and De-Miguel M.[1]

[1] Department of Normal and Pathological Cytology and Histology. School of Medicine. University of Seville, Seville, Spain. [2] Department of Organic and Pharmaceutical Chemistry, University of Seville, Seville, Spain. [3] Gastroenterology Unit, Virgen Macarena University Hospital, Seville, Spain. [4] Department of Medicine, University of Seville, Seville, Spain.

Introduction: Crohn's disease is a chronic autoimmune inflammatory condition of the digestive tract that evolves recurrently with flare-ups and is part of inflammatory bowel diseases (IBD). Crohn's disease could affect the entire digestive tract and is the result of a complex interaction between environmental factors, genetic susceptibility, and the altered gut microbiota, causing dysregulation of innate and adaptive immune responses, and finally producing ROS and inflammation. All this results in a summary of clinical effects such as weight loss, diarrhoea, abdominal pain, and fatigue. In order to reduce the effect of flare-ups in Crohn's disease, in this work we proposed an oral treatment with extract of *Aristotelia chilensis (Ach)*. This plant, which grows in Chile, has a berry-like fruit containing polyphenols with antioxidant and anti-inflammatory properties.

Methods: The effect of Ach was performed in cell culture (HT-29 and RAW264,7) and in a recurrent Crohn model of TNBS in Balb-c mice. Macroscopic data were collected: length and weight of the large intestine, irrigation, feces appearance, presence of mucus and ulcers. Half of the whole colon was rolled and processed for histological sections. Slides were dyed with H&E and PAS to study colon morphology, mucosa integrity, inflammation, etc. On the other hand, sections were used for IHC to study the presence of NLRP6 inflammasome.

Results: Three groups of mice were performed: negative control (no disease), positive control (disease) and Ach (disease + Ach treatment). The macroscopic score showed significant differences between the control positive and control negative/Ach groups, and the control positive showed hyperemia, diarrhea, and in some mice ulcers and the presence of mucus. Furthermore, the length and weight of the large intestine in control-positive mice were lower. H&E showed a distortion of morphology in control-positive mice and the inclusion of immune system cells in the tissue. These characteristics did not appear in the control negative and were less in mice treated with Ach. IHC showed that NLRP6 increased in the positive controls but decreased to control levels after Ach treatment.

Discussion & conclusions: The results demonstrate that Ach improved the damage caused in our Crohn's disease chronic model or at least reduced the effect of flare-ups. Negative control mice and mice from Ach group were similar among themselves, Ach group showed some of the disease symptoms, but was less than in the positive control group. These symptoms were hyperemia or diarrhea; however, they appeared only in the early stages of the experiment.

Supported by CTS-949 (Biopathology and Oxidative Stress Group).

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Histological characterization of the gastrointestinal tract in the maternal immune activation rat model of schizophrenia

Gálvez-Robleño C.[1,2], Pomana B.[1], López-Gómez L.[1,2], Lamanna-Rama N.[4], Romero-Miguel D.[4], Casquero-Veiga M.[8], Desco M.[6,4,5,8], Abalo R.[1,2,3,7], Soto-Montenegro M.L.[4,5,2*] and Uranga-Ocio J.[1,2*]

[1] Dpto. de Ciencias Básicas de la Salud, Universidad Rey Juan Carlos (URJC), Alcorcón (Madrid), Spain; [2] Grupo de Investigación de Alto Rendimiento en Fisiopatología y Farmacología del Aparato Digestivo (NeuGut), URJC, Alcorcón (Madrid), Spain; [3] Unidad Asociada de I+D+i al Instituto de Química Médica, IQM (CSIC), Madrid, Spain; [4] Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain; [5] CIBER de Salud Mental (CIBERSAM), Madrid, Spain; [6] Dpto. de Bioingeniería e Ingeniería Aeroespacial, Universidad Carlos III de Madrid, Escuela Politécnica Superior, Leganés (Madrid), Spain; [7] Grupo de Trabajo de Ciencias Básicas en Dolor y Analgesia de la Sociedad Española del Dolor, Madrid,Spain; [8] Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

Background and aims. Schizophrenia (SCZ) is a complex mental disorder and genetic and environmental studies suggest that alterations in the immune system substantially increase the risk of mental disorder. The gastrointestinal tract (GI) constitutes the largest immune organ in the body and may also be altered in SCZ. The use of the mimetic antigen viral Poly (IC), utilized in the maternal immune activation (MIA) model, produces an SCZ-like phenotype in offspring. Our objective was to characterize the possible pathological alterations developed in the GI tract of this offspring.

Methods. On gestational day 15, Poly I:C (4 mg/Kg) or Saline were injected to pregnant Wistar rats. Male offspring were sacrificed at 90-95 days of age, and samples of the digestive tract (stomach, ileum, distal colon (DC)) were collected for the evaluation of biomolecular and structural alterations.

Results. MIA did not alter the normal histological structure of the stomach (fundus and body) and ileum, but significantly reduced the general damage and their associated Peyer´s patches in the colon and increased the thickness of its muscular layers. Moreover, it did not alter the expression of structural barrier proteins (claudin), proinflammatory enzymes (cyclooxygenase-2, COX-2) or the proportion of some proinflammatory immune cells (mast cells, CD163+ macrophages), neither in ileum nor in DC. However, it did produce an increase in the infiltration of neutrophilic granulocytes, more marked in DC than in ileum, and a statistically significant decrease in CD3+ T lymphocytes in DC.

Conclusions. In male offspring, MIA does not alter the normal structure of the GI tract wall, or integrity of the intestinal barrier, although it increases muscle thickness in DC and may affect motility. It does not seem to favor an inflammatory state through the expression of COX-2, nor an increase in proinflammatory cells that produce fast-acting histamine (mast cells), or in activated proinflammatory macrophages (CD163+). However, in DC, it increases the presence of neutrophilic granulocytes and decreases CD3+ T lymphocytes. This immunological phenotype, indicative of a low-grade colonic neutrophil-dependent inflammation, suggests schizophrenic patients may have a compromised action of the adaptive immune system in the GI tract.

Supported by: Ministerio de Ciencia, Innovación y Universidades [PID2019-111510RB-I00]; Ministerio de Ciencia e Innovación - Instituto de Salud Carlos III [PI17/01766, BA21/00030]; European Regional Development Fund (ERDF) “A way to make Europe”; Delegación del Gobierno para el Plan Nacional sobre Drogas (2017/085); Grupo Español de Motilidad Digestiva (Beca Allergan, 2017).

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Correlation of virulence of different strains of *Helicobacter pylori* with histological parameters

Álvarez-Polanco K.S.[1], Mercado-Sáenz S.[1], Rodríguez Pérez L.M.[1], Sánchez Varo R.[1], Escamilla Sánchez A.[1], Ortega Jiménez M.V.[1,2], Ríos Barranquero M.C.[1], Alba Tercedor M.C.[1], López Villodres J.A.[1] and Bermúdez Flores D.[1]

[1] Medical Biology and Histology PAI Reference: CTS429. Department of Human Physiology, Human Histology, Pathological Anatomy and Physical Sport Education. School of Medicine. Malaga University. Spain; [2] Unit of Anatomical Pathology, Virgen de la Victoria University Hospital, 29010 Malaga, Spain

Introduction: Helicobacter pylori (H. pylori) is a gram-negative bacillus with a spiral shape, which has between 2 and 6 monopolar flagella, whose ideal habitat is the human gastric mucosa at the level of the mucus layer that covers the gastric cells, and with special predilection for the antral mucosa. The colonization of this bacterium gives rise to chronic gastric inflammation that can lead to gastritis, peptic ulcer, gastric cancer and mucosa-associated lymphoma, changing its histology. H. pylori has different adaptive mechanisms, including urease, flagella, outer membrane proteins, and a type IV secretion system, responsible for releasing toxins responsible for cell damage.
Hypothesis: Is it possible that different strains of the bacteria produce changes in normal gastric histology?
Objectives: - Know the general characteristics of Helicobacter Pylori. - Pathogenesis of H. Pylori. -Determine the virulence factors and their ability to cause damage to the host. -Study on the different strains. - Correlation between the normal histology of the stomach and the changes derived from the colonization of different strains.
Method: For this review, the PubMed database has been used. It is a solid database, with free access, which has prestige in the health area, being mostly scientific publications from the MEDLINE database.The first bibliographic search was carried out in English with its equivalents in Spanish, of original articles in English from the last 10 years, using descriptors for it (Helicobacter pylori, VacA, CagA, virulence, histology, strains, virulence marker). 91 of the 190 articles that have appeared in relation have been selected, the rest have been discarded because they are not directly related to the content of this review and many others because they are private paid articles. In the second bibliographic search, new key words in English were included with their equivalents in Spanish, such as (MALT lymphoma, ulcer, gastritis, gastric cancer) with the aim of developing the diseases caused by H. pylori and their respective histological changes. Due to the large number of articles found and in order to keep it current, reviews of the last 5 years have been used. 12 articles of the 231 that have appeared in relation have been selected, the rest have been discarded for not having a direct relationship with the subject.
In the sections that include histological descriptions, the 6th edition of the book "Histology: Text and Color Atlas with Cellular and Molecular Biology" whose authors are Michal H. Ross and Wojciech Pawlina, of the Editorial Médica Panamericana, has been used. It has been selected because it is the reference text par excellence in the field of histology, presenting detailed and high-quality illustrations and color photographs accompanied by their respective descriptions, being very useful to understand the global organization of the topic to be treated in this review. In the peptic ulcer section, the book "Histology with functional and clinical correlations" published by Lippicott Williams & Wilkins and whose author is Dongmei Cui, has also been used.
Results: Information was obtained on Helicobacter pylori, its characteristics, virulence strains associated with genes such as: Gen Saba, Gen VacA, Gen CagA, Lipopolysaccharide (LPS) Antigens, Western CagA, East Asian Cag, MALT lymphoma, ulcer, gastritis, gastric cancer.
It can be determined that Helicobacter pylori is a bacterium that affects 35-70% of the underdeveloped population. At the time colonization by H. pylori occurs, the structural changes suffered by the foveolae that make up the gastric columnar epithelium are observed in histological sections. The main virulence factors studied are those involved in inflammation and cell damage, particularly those encoded in the cag pathogenicity island (CagPAI) as well as other proinflammatory proteins.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Evolution of grading criteria in Central Nervous System neoplasms: bridging the gap between morphology and molecular biology. A case report

Gil-Belmonte MJ.[1,2], Maldonado-Berrios A.[2], Velasco-Albendea F.J.[1], Jimena I.[2,3] Berenguel-Ibáñez M.[1] and Pérez-Rodríguez A.[1]

[1] Department of Pathology, Torrecárdenas University Hospital, Almería, Spain; [2] Department of Morphological Sciences, Section of Histology, Faculty of Medicine and Nursing, University of Córdoba, Spain; [3] Maimonides Institute for Biomedical Research IMIBIC, Reina Sofia University Hospital, University of Córdoba, Spain.

Background: First published in 1997, the WHO has been trying to typify each neoplastic lesion affecting the brain and spinal cord in its Classification of Central Nervous System Tumors. Although previous grouping attempts were based on histological features, the development of molecular biology has progressively allowed the integration of molecular and morphological characteristics in daily histopathological practice. Today, 2021 WHO Central Nervous System Tumor Classification, goes one step beyond giving biomarkers a preponderant role in a hybrid taxonomy that exemplifies the current state of the field, likely, only an intermediate stage to an even more precise future classification.

Methods: A 55-year-old woman presenting with neurological deficits was diagnosed with a growing mass affecting the right frontal lobe. After surgical excision and its prior intraoperative biopsy, a formalin-fixed sample of encephaloid tissue was submitted to our Pathology service for its analysis. A routine hematoxylin-eosin study was made supported by immunohistochemical techniques for IDH-1, ATRX, p53, and proliferative Ki67 index. Once a presumption diagnosis was released, paraffin-embedded blocks were sent to an external service to determine the CDKN2A/B homozygous deletion status to establish an adequate grade according to the reference guidelines.

Results: Morphologically, tumor cells resembled that of a glial-precursor neoplasm. One single anaplasia spot and areas of incipient and subtle microvascular proliferation were found, whereas no necrosis or mitoses were observed. Immunohistochemical techniques revealed an IDH-mutant status and an ATRX loss. Alterations in p53 were also detected, and Ki67 scored approximately a 10%. Given that, the first diagnostic approach was "Astrocytoma, IDH-mutant, grade 3". Then, remnant tissue was sent for FISH analysis, which evidenced a CDKN2A/B homozygous deletion, highlighting the need to upgrade the tumor to grade 4.

Discussion & Conclusions: As the utility of molecular biomarkers for neuropathological diagnoses has been further elucidated, challenges have arisen in how to diagnose and grade the different tumors of the Central Nervous System. An example of this can be observed in astrocytomas: IDH-mutant neoplasms ranging between grades 2 to 4. The assessment of histopathological grade for these neoplasms depends on histoarchitectural features such as necrosis or manifest microvascular proliferation. However, performing molecular analysis for CDKN2A/B is mandatory for ruling out a grade 4 in astrocytomas, even if histological findings seem of a low-grade diffuse astrocytoma, meaning items of critical prognostic relevance. Because of the growing importance of molecular information, diagnoses and diagnostic reports need to combine different data types into a single, “integrated” diagnosis. The relevance of correctly establishing a diagnosis relies upon the progressive development of new therapies against concrete molecular targets. We are undoubtedly at a time of crucial changes in the world of neuropathology. The fusion between histological features and molecular determinations opens the door to precision medicine, allowing us to advance in the name of knowledge.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Histopathological study of glycolytic activity in patients with chronic venous disease

Fraile-Martinez O.[1,2*], Ortega M.A.[1,2], Garcia-Montero C.[1,2], Guijarro L.G.[2,3], Saez M.A.[1,2,4], Ruiz-Grande F.[5], Monserrat J.[1,2], Alvarez-Mon M.[1,2,6], García-Honduvilla N.[1,2] and Bujan J.[1,2]

1Universidad de Alcalá, Department of Medicine and Medical Specialties, Faculty of Medicine and Health Sciences, 28801 Alcalá de Henares, Spain. 2Ramón y Cajal Institute for Health Research (IRYCIS), 28034 Madrid, Spain. 3University of Alcalá, Unit of Biochemistry and Molecular Biology, Department of Systems Biology (CIBEREHD), 28801 Alcalá de Henares, Spain 4Service of Pathological Anatomy, Hospital Universitario Central de la Defensa-UAH, 28001 Madrid, Spain. 5Vascular Surgery Department, Hospital de la Princesa, 28834 Madrid, Spain. 6Department of Internal Medicine and Diseases of the Immune System-Rheumatology, Oncology (CIBEREHD), Hospital Universitario Príncipe de Asturias, 28806 Alcalá de Henares, Spain. *Correspondence author: oscarfra.7@hotmail.com

Chronic venous disease (CVD) is a widely represented medical condition in our society, characterized by a series of structural and functional abnormalities of the venous system. Varicose veins (VVs) represent a frequent clinical manifestation of CVD, especially in the lower extremities. Previous histopathological studies have defined a set of alterations observed in the venous wall of patients with VV, negatively affecting its composition and behavior. Metabolic changes in the veins appear to be a critical biological mechanism that has helped to understand the pathogenesis of CVD. In this sense, previous studies have identified a potential role of the glycolytic phenotype in the development of different vascular disorders; however, his precise role in EVC has yet to be fully explored. Thus, the objective of the present study was to determine the gene and protein expression of the glucose transporter 1 (GLUT-1) and of the glycolytic enzymes PGK-1, ALD, GA3PDH and LDH in the VVs of patients with CVD (N=35). compared to those expressed in healthy subjects (N=27). Our results show a higher histopathological expression of GLUT-1, PGK-1, ALD, GA3PDH and LDH in subjects with CVD, which indicates how the venous tissue has a higher glycolytic activity, with possible pathophysiological implications. Therefore, the glycolytic phenotype observed in VVs may represent a potential therapeutic target in these patients, also opening future paths that explore the impact of this glycolytic change in patients with CVD.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

EBV-positive Gastric Cancer. Determination and Clinicopathological Implications. A pilot study

Parra G.[1], Arranz-Salas I.[1,2], Ortega M.V.[2,3], Mercado S.[1], Sánchez-Varo R.[1], Escamilla A.[1], Rodríguez-Pérez L.M.[1], Hierro I.[2], López-Villodres J.A.[1] and Bermúdez D.[1]

[1]Department of Human Physiology, Human Histology, Anatomical Pathology and Physical Education. University of Malaga, 29010 Malaga, Spain;[2] Unit of Anatomical Pathology, Virgen de la Victoria University Hospital, 29010 Malaga, Spain; [4] Unit of Radiation Oncology, Virgen de la Victoria University Hospital, 29010 Malaga, Spain

Introduction. Gastric cancer (GC) is the fifth most common malignant neoplasm worldwide in terms of incidence in both sexes. It is also in third position in terms of mortality, considered because of late diagnosis and ineffective treatment. One of the risk factors for its appearance is the infection by Epstein-Barr virus (EBV), which is included in several biomolecular classifications of GC, such as The Cancer Genome Atlas. As shown in a previous study the combination of new molecular classifications with clinicopathological data could contribute to the individualization of patients and to the development of new therapeutic strategies. The aim of this study was the implementation of the technique (not routinely performed in our hospitals) and correlate the presence of EBV infection with clinicopathological features and other biomolecular data of the patients previously identified, such as HER2 status (Human Epidermal Growth Factor Receptor 2) and microsatellite instability (MSI).

Methods. A retrospective study of 53 GC patients was performed. The presence of EBV infection was determined by in situ hybridization. Clinicopathological data considered were as follows: age, sex, location, pathological diagnosis, histological type according to the Lauren classification, degree of differentiation, HER2 and microsatellite instability determination, TNM classification and stage, lymphatic, vascular and perineural involvement, perioperative chemotherapy, and survival.

Results. The general clinicopathological features of our patients were as follows: age around 65 years, male sex, location in the gastric body, diagnosis of intestinal adenocarcinoma, with poorly differentiated cells and high tumor stage; lymph node involvement was frequently found, in contrast to distant metastases. Regarding the previously determined biomolecular characteristics, 5.66% HER2-positive patients and 9.43% MSI patients were found. Three patients tested positive for EBV identification (5.66%), showing lower than expected percentages for all three GC types. A statistical analysis was carried out, and no significant correlations were obtained, probably due to the limited sample size. Nevertheless, trends in the features of the EBV-positive individuals were compared with those reported in the literature. A predominance of male sex and advanced age was observed, as well as low tumor stages with rare lymph node involvement and few distant metastases. Furthermore, EBV-positive GC has been associated with HER2 and MSI negative types, and low survival. In conclusion, larger studies are needed to obtain results with greater statistical confidence and apply them to clinical practice.

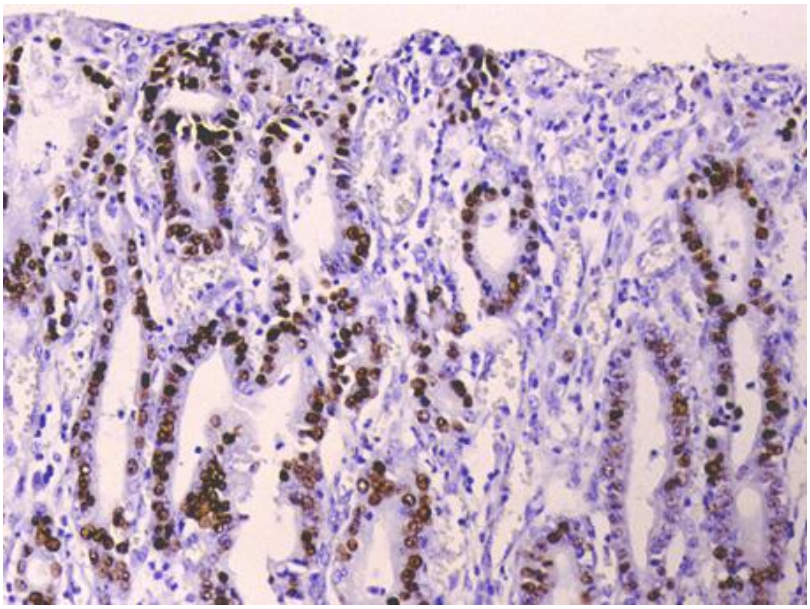


Figure 1. EBV-positive gastric cancer. In situ hybridation to EBV-encoded RNA EBER1 and EBER2 (400x)

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Diagnostic value of p53 immunohistochemical expression in high-grade serous ovarian carcinoma (HGSC): casuistry in our center in the last three years

Ortega Jiménez M.V.[1,2], Díaz Baena C.[1,2], Hierro Martín M. I.[1,2], Arranz Salas I.[1,2], Rodríguez Pérez L.M.[1], Mercado Sáenz S.[1], Alba Tercedor M.C.[1], López Villodres J.A.[1] and Bermúdez Flores D.[1]

[1] Department of Histology, University of Malaga, Malaga, Spain; [2] Department of Pathology, University Hospital Virgen de la Victoria, Malaga, Spain; [2] Unit of Anatomical Pathology, Virgen de la Victoria University Hospital, 29010 Malaga, Spain

Background: High-grade serous carcinoma (HGSC) is the most common ovarian carcinoma (70%). Age over 60 years, a family history of breast/ovarian carcinoma, and infertility are risk factors. Etiology remains unknown. These tumours arise from tubal-type epithelium, usually in the fallopian fimbria and less commonly in the ovarian surface or within ovarian epithelial inclusion cysts. Nearly every tumour harbours a deleterious TP53 mutation [1]. P53 immunohistochemistry helps to distinguish high-grade serous from low-grade serous carcinomas, which is essential given the differences in management: primary treatment (possible neoadjuvant platinum-based chemotherapy for high-grade serous vs. upfront surgery for low-grade serous carcinomas), adjuvant therapies [poly (ADP-ribose) polymerase inhibitors for BRCA1/2-mutated high-grade serous vs. hormonal therapy for low-grade serous carcinomas] and future targets (CCNE1 amplification for high-grade serous vs. CDKN2A loss in low-grade serous carcinomas [2].
Methods: A review of all cases diagnosed in our centre of high-grade serous ovarian carcinoma in surgical specimens, between 1 January 2019 and 31 December 2021, was carried out. The immunohistochemical profile (WT-1, p16 and p53) was compared with the results obtained in the massive NGS sequencing panel, focusing on the study of p53. Among the diagnosed cases, the result of the p53 mutational status obtained by immunohistochemical technique (non-mutated, mutated with overexpression, mutated with null pattern or absence of staining) was correlated with the result obtained by sequencing technique (Oncomine, NGS).
Results: In our centre, 32 cases of high-grade ovarian serous carcinoma were diagnosed in 2019, 2020 and 2021, of which 31 showed a mutates immunohistochemical staining pattern (65% with p53 overexpression and 32% with null pattern), and 1 a non-mutated pattern. Of these 31 patients, 19 (61%) underwent genetic sequencing (Oncomine). Of these 19 patients, 16 (84%) had a p53 somatic lineage mutation and 3 (16%) were non-mutated. Immunostaining was positive for p16 in 30 cases, with intense cytoplasmic and nuclear staining expression in 27 of them and patchy and diffuse in 3, WT1 was positive in all of them.
Discussion and conclusions: In this case series we would like to share our centre´s case reports on this pathology, with special emphasis on the importance of p53 immunohistochemical staining as a surrogate marker for sequencing techniques. Its use allows patients to be classified into high or low grade serous carcinomas, important in biopsy for diagnostic and therapeutic orientation due to its high degree of sensitivity to chemotherapy treatment in those with mutation, making this marker one of the most relevant in ovarian tumour pathology.

[1] Who Classification of Tumours Editorial Board. (2020). Female Genital Tumours: Who Classification of Tumours (5th ed.). World Health Organization. 45-47
[2] Köbel M. and Kang E. (2020). The Many Uses of p53 Immunohistochemistry in Gynecological Pathology: Proceedings of the ISGyP Companion Society Session at the 2020 USCAP Annual 9 Meeting. Int. J. Gynecol. Pathol. 40, 32-40

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Hepatic perivascular epithelioid cell tumor (PEComa) mimic an adenoma

Arranz-Salas I.[1,2], Escalona-Garcia A.[1,2], Hierro Martín M.I.[1,2], Ortega Jiménez M.V.[1,2], Rodríguez Pérez L.M.[1], Sánchez Varo R.[1], Escamilla Sánchez A.[1], Ríos Barranquero M.C.[1], Mercado Sáenz S.[1], López Villodres J.A.[1] and Bermúdez Flores D.[1]

[1] Department of Histology, University of Malaga, Malaga, Spain; [2] Department of Pathology, University Hospital Virgen de la Victoria, Malaga, Spain; [2] Unit of Anatomical Pathology, Virgen de la Victoria University Hospital, 29010 Malaga, Spain;[4] Unit of Radiation Oncology, Virgen de la Victoria University Hospital, 29010 Malaga, Spain

Background: PEComas are tumors of mesenchymal origin characterized histologically by both the proliferation of perivascular epithelioid cells and the ability to co-express melanocytic and smooth muscle immunohistochemical markers.We present the case of a patient with an incidental finding of hepatic LOE on abdominal CT radiologically compatible with hepatic adenoma and who, after resection and anatomopathological study, was diagnosed with PEComa (monotypic epithelioid angiomyolipoma).
Methods: A 48-year-old woman, asymptomatic and with resolved CVH as the only history of interest, in who solid hepatic lesion in the caudate lobe of 6.8x5.5 cm compatible with adenoma was detected on an abdominal CT. A surgical approach was decided, carrying out a limited hepatic reseccion.
Results: Macroscopically, a 6x5 cm yellowish lesion with foci of a congestive-hemorrhagic appearancewasobserved.
The microscopic study revealed a heterogeneous tumor formed by large vessels, foci of adipose tissue, solid pattern areas and scattered areas with a pleomorphic appearance predominantly located in the perivascular area. At higher magnification, epithelioid cells with clear cytoplasm and nuclei with evident nucleoli predominated. No mitoses were observed.
Tumor cells strongly and diffusely immunoexpressed Actin, Caldesmón, HMB45 and MelanA; the expression of Desmin, Ckit, TFE3-, CKAE1AE3-, Hepar- and Glipican- being negative.
Therefore, the tumor showed absence of epithelial and hepatocyte markers as well as positivity for smooth muscle markers. Although at first the possibility that it was a muscular neoplasm was assessed, the absence of expression of a specific smooth muscle marker such as Desmin, together with the finding of foci of adipose tissue in the neoplasm, were key to the diagnosis. thus adding to the immunohistochemical study the melanocytic markers (HMB45 and Melan-A) that were positive. The combination of the histological and immunohistochemical findings, as well as the low proliferative index despite the cellular pleomorphism that it presented, confirmed the diagnosis of hepatic PEComa (Angiomyolipoma type).
Conclusions: PEComas are rare mesenchymal neoplasms that predominantly occur in middle-aged women. The most frequent location is the uterus followed by the kidney and they can be associated with tuberous sclerosis. The clinic is variable although they are generally asymptomatic.
The diagnosis of PEComa before surgery is very difficult due to the similarity of its appearance in imaging tests (Rx) with other liver lesions, mainly adenoma. Its diagnosis is almost always incidental in the surgical piece, since it does not cause symptoms.
Most PEComas are considered benign. However, there are certain histological characteristics that make some subgroups of lesions suspicious for malignant transformation, so surgical resection of the lesion with free margins is recommended, in addition to close follow-up to detect possible recurrences or other complications.

[1] Who Classification of Tumours Editorial Board. (2020). Soft Tissues Tumours: Who Classification of Tumours (5th ed.). World Health Organization. 45-47
[2] J. Clin. Med. 2021, 10, 1756. https://doi.org/10.3390/jcm10081756

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

PCSK9 and CD133 coexpression in colorectal cancer stem cells

Crende O.[1], Martin A.[2], Garcia H.[2], Badiola I.[2] and Garcia-Gallastegi P.[2]

[1] Department of Cell Biology and Histology, Faculty of Pharmacy, University of the Basque Country, Vitoria-Gasteiz 01006, Araba, Spain; [2] Department of Cell Biology and Histology, Faculty of Medicine and Nursery, University of the Basque Country, UPV/EHU, Leioa 48940, Bizkaia, Spain.

Introduction: Protein Convertases are serine proteases responsible for maturing inactive proproteins. Up to now, nine types of convertases have been described: PCSK1, PCSK2, FURIN, PCSK4, PCSK5, PCSK6, PCSK7, SKI1 and PCSK9, whose functions are varied and, to a great extent, essential [1]. Therefore, any change in its expression affects homeostasis, generating pathologies. Their involvement in the activation of adhesion molecules, metalloproteinases and proinflammatory molecules has been described, important in tumoral development, the implications of convertases have been studied in many tumors. Subtilisin/kexin type 9 (PCSK9) has been described as key regulator in levels of LDL cholesterol. This convertase is mainly expressed in the liver and its effect in tumoral development and liver metastasis is being investigated [3].
Our study aimed to further elucidate the role of PCSK9 in cancer, and more specifically in Cancer Stem Cells (CSC)s, that have been related to be responsible for cancer initiation, progression, metastasis, recurrence and drug resistance.
Methods: Previously, we demonstrated a different pattern of gene-expression and protein levels of PCSK9 in parental cells and CSCs from metastatic and non-metastatic human colorectal adenocarcinoma cells lines. In addition, PCSK9 inhibition significantly decreased CSC surface area and viability. Now we analyze by immunofluorescence the co-expression of PCSK9 and CD133 (CSC marker) in CSCs from metastatic and non-metastatic human colorectal adenocarcinoma cells lines and after inhibition of PCSK9 expression. Then, expression of these two markers were studied in biopsies of primary and metastatic colorrectal cancer.
Results: We found a higher expression and a colocalization of PCSK9 and CD133 in CSCs from metastatic and non-metastatic human colorectal adenocarcinoma cells lines compared to parental cell lines. In the other hand, the percentage of colocalization of PC9 and CD133 was higher in liver metastasis than in primary tumors.
Discussion & conclusions: The colocalization between PCSK9 and stem cell marker in CSC in cell lines and with greater colocalization in metastases, suggested the existence of a correlation between PCSK9 expression and CSC of the colorectal cancer. PCSK9 may play a role in tumor development and metastasis.

[1] Seidah NG and Prat A. Nature Reviews Drug Discovery. 2012;11(5):367–383
[2] Seidah NG, et al. International Journal of Biochemistry and Cell Biology. 2008;40(6–7):1111–1125.
[3] Sun X, et al. Neoplasia. 2012;14(12):1122–1131

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Prognostic value of IRS-4 as a histopathological biomarker in pancreatic cancer

Garcia-Montero C.[1,2*], Ortega M.A.[1,2], Fraile-Martinez O.[1,2], Saez M.A.[1,2,3], Monserrat J.[1,2], Alvarez-Mon M.[1,2,4], García-Honduvilla N.[1,2], Guijarro L.G.[2,5] and Bujan J.[1,2]

1Universidad de Alcalá, Department of Medicine and Medical Specialties, Faculty of Medicine and Health Sciences, 28801 Alcalá de Henares, Spain. 2Ramón y Cajal Institute for Health Research (IRYCIS), 28034 Madrid, Spain. 3Department of Pathological Anatomy, Hospital Universitario Central de la Defensa-UAH, 28001 Madrid, Spain. 4Department of Internal Medicine and Diseases of the Immune System-Rheumatology, Oncology (CIBEREHD), Hospital Universitario Príncipe de Asturias, 28806 Alcalá de Henares, Spain. 5Universidad de Alcalá, Unit of Biochemistry and Molecular Biology, Department of Systems Biology (CIBEREHD), 28801 Alcalá de Henares, Spain *Corresponding author: cielo.gmontero@gmail.com

Pancreatic cancer is a malignant neoplasm with a growing incidence, especially in developed countries due to causes such as sedentary lifestyle, smoking and ultra-processed diets rich in fats and refined sugars, among others. In fact, it is the seventh leading cause of cancer-related death worldwide and, in the coming years, it is expected to climb to second position, after lung cancer. This is due to the fact that this type of tumor can have an asymptomatic course, manifesting itself in advanced stages, generally when there has already been metastasis. In addition to the low survival rates that this type of tumor presents, there is a high possibility of recurrence in patients who survive it. The identification of new molecular biomarkers is emerging as a very useful tool for the clinical management of pancreatic cancer, although there is still much research and work to be done in this field. Thus, the present study aims to analyze a series of molecules such as the insulin receptor substrate 4 (IRS-4), the retinoblastoma protein (Rb1), the Ki-67 marker and cyclooxygenase 2 (COX-2) as candidates to prognostic biomarkers. Thus, its histopathological expression was analyzed using immunohistochemical techniques with a 60-month longitudinal surveillance program, associated with various clinical parameters. The Kaplan-Meier curves that estimate the survival time according to the tumor expression of these molecules denoted a low cumulative survival rate in the patients analyzed (N=41). Of all of them, high levels of IRS-4 were the most significantly associated with a poor prognosis of the disease, increasing the risk of mortality 160 times. In this way, our research showed a relevant value of these biomarkers in the survival of patients with pancreatic cancer, especially IRS-4, opening a potential therapeutic window for future studies.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Quantification of Ki-67 in cervical cancer through digital image analysis improves interobserver and intraobserver concordance

Cruz-Ramos J.A.[1,2], Ramos-Márquez M.E.[1,2], López-Armas G.C.[3], Arias-Novoa E.[1], Correa-Santillan V.M.[1], Farias-López M.[1] and Falconi-Olán E.[1]

[1] Instituto Jalisciense de Cancerología, Guadalajara, Jalisco, México; [2] Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, México; [3] Centro de Enseñanza Técnica Industrial, Guadalajara, Jalisco, México;

Introduction: IHC markers, like Ki-67 may predict survival, risk of recurrence and response to treatment in cervical cancer (CuC), these markers are intended to optimize the management of the disease. Ki-67 protein expression is an indicator of an active cell cycle (G1, S, G2 and mitosis). The analysis and grading of the expression of IHC markers with conventional manual methodologies is highly subjective, the reproducibility is low even by trained personnel, so it is necessary to implement methodologies that provide greater precision in the quantification of IHC markers. The emergence of digital pathology has led to innovative methodologies for analyzing and quantifying the expression of IHC markers in biological samples, based on algorithms and macros to automate or semi-automate the process.

Methods: Cross-sectional correlational study. CuC samples were obtained from the pathology archive and IHC for Ki-67 was performed. Quantification of Ki-67 was performed manually and with ImageJ software assistance to determine the variability and concordance of the 2 methodologies. Intraobserver and interobserver comparisons were performed with Student's t-test, Pearson's correlation test and Bland-Altman plots.

Results: The results obtained from manual and software evaluations with the Student's t-test did not show significant differences (p>0.05) between observers. The intraobserver and interobserver Pearson correlation was statistically significant (p<0.05) within and between the 3 observers. The correlation between the observer using software and both observers with manual technique was significant (p<0.05). The values obtained by the software-assisted method had the highest uniformity and highest correlation (r2=0.95, p<0.0001) of all the evaluations, with robust linearity, covering a large number of intermediate values, not accumulating at the extremes as in the observers with the manual method.

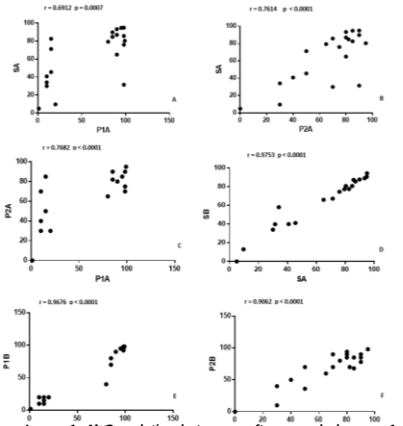


Image 1. A) Correlation between software and observer 1; B) Correlation between software and observer 2; C) Correlation between observer 1 and observer 2; D) Intraobserver correlation software-software E) Intra-observer correlation, observer 1 and F) Intrainterobserver correlation, observer 2.

Discussion & conclusions: The comparisons made in this research show that the use of software-assisted method in the quantification of Ki-67 in CuC decreases the variability in the evaluation of the percentage of stained nuclei, and the correlation with the manual method is significant (p<0.05). The correlation between software-assisted quantification and manual quantification, although it is statistically significant, r2 is less than 60. According to the literature, in this research it was demonstrated that the correlation between different histopathologists is low, r2=0.59. These observations suggest that the use of digital analysis of ki-67 images in CuC may be useful to increase the accuracy of quantification and thus better predict clinical outcomes during CUC treatment.

[1] Silva DC., Gonçalves AK., Cobucci RN., Mendonça RC., Lima PH. and Cavalcanti G. (2017). Immunohistochemical expression of p16, Ki-67 and p53 in cervical lesions - A systematic review. Pathol Res Pract 213(7):723-729.

[2] Konsti J., Lundin M., Joensuu H., Lehtimäki T., Sihto H., Holli K., et al. (2011). Development and evaluation of a virtual microscopy application for automated assessment of Ki-67 expression in breast cancer. BMC Clin Pathol 11(1):3.

[3] Samarawardana P., Singh M. and Shroyer KR. (2011) Dual stain immunohistochemical localization of p16INK4A and ki-67: a synergistic approach to identify clinically significant cervical mucosal lesions. Appl Immunohistochem Mol Morphol 19:514-8.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Retrospective descriptive study of injuries in prophylactic annexectomies of patients with mutation in BRCA gene

Ortega Jiménez M.V.[1,2], Ramírez Sánchez C.[1,2] and Hierro Martín M. I.[1,2], Arranz Salas I.[1,2], Escamilla Sánchez A.[1], Sánchez Varo R. [1], Ríos Barranquero MC.[1], Mercado Sáenz S.[1], López Villodres JA.[1] and Bermúdez Flores D.[1]

[1] Department of Histology, University of Malaga, Malaga, Spain; [2] Department of Pathology, University Hospital Virgen de la Victoria, Malaga, Spain; [2] Unit of Anatomical Pathology, Virgen de la Victoria University Hospital, 29010 Malaga, Spain

Background: Risk Reduction Salpingo Oophorectomy (RRSO) is performed in women carriers of the BRCA gene mutation due to the increased risk they present of developing ovarian cancer (mainly high-grade serous type), this being higher in BRCA1 mutations. These mutations predispose to have other types of cancer, such as breast cancer. In about 10% of Intraepithelial neoplasias are evident in the tubes of these RRSOs, being the most of them precursors of tubal intraepithelial carcinoma (STIC), such as called *Signal p53*, which is defined, according to the Kurman algorithm, as a secretory proliferation presenting at least 12 reactive epithelial cells for p53 and ki67<10%.

Material and Methos: A retrospective descriptive study of women carriers of BRCA in our center in which RRSO had been performed in an interval of 5 years; since from 03-28-2017 to 03-28-2022. The search returned 58 prophylactic adnexectomy, in which p53 and ki67 biomarkers were analyzed. A detailed search of epidemiological data: age, type of BRCA, history relatives of breast or ovarian cancer, as well as a personal history of breast cancer. For the diagnostic management of the histological samples, the serous tubal intraepithelial carcinoma algorithm by Kurman et al., validated in 2012, based on the combination of histological features and immuno-histochemical nuclear expression of p53 and Ki-67.

Results: Of the 58 patients operated on for RRSO, 24 had the BRCA1 mutation and 34 the mutation in BRCA2. After an exhaustive review, 4 cases (7%) were found withp53 signal and no cases of STIC. Of these 4 cases of Signal p53, 3 presented mutation in the BRCA2 gene and only 1 in BRCA1. Regarding epidemiological data, 2 of these patients had breast cancer as a personal history. Of the entire sample, 20 patients (34%) had a family history of breast and/or ovarian cancer, of which 1 presented p53 signal; and 16 patients (27%) presented both family and personal history, of which 1 (6%) presented p53 signal. Only 9 women (15%) did not present family or personal history, nor tubal injury.

Conclusions: Women who carry mutations in the BRCA1 or BRCA2 genes have an elevated risk of developing ovarian cancer during her lifetime. They recommend SORR at the time of satisfied parity, almost always between 35 and 40 years of age for BRCA1 and between 40 and 45 years for BRCA2.Immunohistochemistry for Ki-67 and p53 was performed in all patients. So we fit all the studied tubes into 4 categories: normal, STIC, STIL (Intraepithelial Tubal Lesion), within which we find the p53 Signal type. In our series we have obtained 4 cases with diagnoses different from normal: 4 p53 signals. This study reveals the existence, in a non-negligible amount (7%) of an initial tubal injury that reflects, in the most cases, a mutation of the p53 tumor suppressor gene, which could play an important role in the tumorigenesis of serous ovarian carcinoma.

Vang R, et al. Validation of an algorithm for the diagnosis of serous tubal intraepithelial carcinoma. Int J Gynecol Pathol. 2012 May;31(3):243-53. doi: 10.1097/PGP.0b013e31823b8831. PMID: 22498942; PMCID: PMC3366037

Munakata, Satoru M.D., et al. Incidence of Serous Tubal Intraepithelial Carcinoma (STIC) by Algorithm Classification in Serous Ovarian Tumor Associated with PAX8 Expression iTubal Epithelia, International Journal of Gynecological Pathology: January 2015 - Volume 34

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Studying the significant prognostic information of tumor associated macrophages (TAM) in breast cancer

Durán E.[1] and Arriazu R.[1]

[1] Histology Laboratory, Institute of Aplied Molecular Medicine, Department of Basic Medical Sciences, School of Medicine, CEU San Pablo University, Madrid

Introduction: Tumour microenvironment is a complex structure which consists of a dynamic mixture of cells. . The most common features of cancer-related inflammation include tumor-associated macrophages (TAMs) this types of cells are closely involved in tumorigenesis by enhancing tumor proliferation, invasion, and metastasis. It is associated with poor prognosis of solid tumors. [1] CD68 is considered the best marker protein TAMs. [2] Macrophages are a heterogeneous cell population that, currently, is divided into two subgroups: the classically-activated type 1 macrophages (M1), which play important roles in the innate response against pathogens and produce large amounts of pro-inflammatory cytokines such as IL-1β; and the alternatively-activated type 2 macrophages (M2), that participate in tissue repair and wound healing, regulation of immunity and tumor progression, and produce anti-inflammatory cytokines, for instance, IL-10. [3, 4] The aim of this study was to investigate the relationship between TAMs, including their phenotypes (M1 and M2), and clinicopathological variables in breast cancer, as well as the prognostic role of TAMs in breast cancer.
Methods: Immunohistochemically expression of Ki67, CD68, IL-1β, IL-10 and CD34 were measured.
Ki67, CD68, IL-1β, IL-10 and CD34 immunopositive cells were counted, and the results are shown as a number of cells or a number of the total vessel fractions. The associations between CD68, IL-1β, IL-10 and CD34 expression and clinicopathological variables were evaluated with the Mann-Whitney U test and the Kruskal-Wallis test. Patients were divided into 4 groups based on molecular features: Luminal A, Luminal B, Her2 and Basal types.
Data normality was tested by Shapiro-Wilk test. The Kaplan-Meier method, log-rank testing, and Cox proportional hazards regression models were used to analyze the association between immunoexpression and outcome with the SPSS software. Results: All patients were female, with a median age of 50 years old at the time of diagnosis. 73.3% breast cancer population were grade 2; The 26.7% of the patients were Luminal A breast cancer.
All the patients were distributed according to whether the tumour had metastasized into No (non-metastatic tumour), 78.3%; and Yes (metastatic tumour), 21.7%. Expression of CD68, IL-1β, IL-10 within the tumor showed cytoplasm staining patterns. High numbers of $CD68^+$ macrophages and $IL\text{-}10^+$ macrophages M2 marker were significantly associated with risk of progression.
Discussion & conclusion: When we studied the possible prognostic role of macrophages markers alone, we observed that the macrophage infiltration was significantly higher in tumors with poor differentiation and tumor metastasis than in tumors with well differentiation and non-metastatic status. These results suggesting that elevated inflammatory responses in the tumor microenvironment are important for malignant progression. In conclusion, high expression of TAMs (overall macrophages, M1- and M2- phenotypes) were significantly correlated with the molecular subtypes of breast cancer and metastatic status. Its expression were associated with a poor survival in two macrophages markers (CD68 and IL-10).

[1] Baghban, R., Roshangar, L., Jahanban-Esfahlan, R. *et al.* Tumor microenvironment complexity and therapeutic implications at a glance. *Cell Commun Signal* **18,** 59 (2020)
[2] Chistiakov, D., Killingsworth, M., Myasoedova, V. *et al.* CD68/macrosialin: not just a histochemical marker. *Lab Invest* **97,** 4–13 (2017)
[3] Moratal, C., Raffort, J., Arrighi, N. *et al.* IL-1β- and IL-4-polarized macrophages have opposite effects on adipogenesis of intramuscular fibro-adipogenic progenitors in humans. *Sci Rep* **8,** 17005 (2018)
[4] Qi, L, Yu, H, Zhang, Y, Zhao, D, Lv, P, Zhong, Y, & Xu, Y. IL-10 secreted by M2 macrophage promoted tumorigenesis through interaction with JAK2 in glioma. *Oncotarget* **1;7** 71673-71685 (44) (2016)

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Gastrointestinal Stromal Tumor (GIST) and its relationship with germline mutations

Arranz-Salas I.[1,2], Jiménez-Campillo M.[1,2], Hierro-Martín M.I.[1,2], Ortega Jiménez M.V.[1,2], Mercado Sáenz S.[1], Rodríguez Pérez L.M.[1], Sánchez Varo R.[1], Escamilla Sánchez A.[1], Peláez González A.[1] and Bermúdez Flores D.[1]

[1] Department of Histology, University of Malaga, Malaga, Spain; [2] Department of Pathology, University Hospital Virgen de la Victoria, Malaga, Spain; [2] Unit of Anatomical Pathology, Virgen de la Victoria University Hospital, 29010 Malaga, Spain

Background: We present the case of a 38-year-old man with a history of abdominal paraganglioma 10 years ago, who consulted for hematemesis and asthenia of 5 days' evolution. An upper gastrointestinal endoscopy was performed where a raised submucosal lesion, about 2 cm, with ulceration on its surface, was observed at the corporal-antral junction. The CT scan revealed nodular thickening of the gastric wall at the level of the lesser curvature. After the resolution of his hematemesis, it was decided to intervene on the patient, performing a partial gastrectomy.

Methods: The piece presented a tumor lesion with a maximum diameter of 1.3 cm. When cut, the tumor was whitish in color and had an elastic consistency. Histologically, it was a mesenchymal neoplasm of epithelioid cellularity that infiltrated the entire thickness of the wall, sparing the mucosa. The growth pattern was multinodular with plexiform infiltration of the muscularis propria layer. The mitotic index was > 5/50 CGA and images of vascular permeation were observed. The immunohistochemical study showed positivity for CKIT, DOG-1 and CD-34. With the diagnosis of Gastrointestinal Stromal Tumor (GIST), we proceeded to carry out the mutational study that resulted in CKIT and PDGFR Wild Type (WT).
Given the morphological characteristics and the immunohistochemical and molecular findings, we proceeded to study the presence of succinate dehydrogenase (SDH), observing a deficit of the B subunit of SDH, with the SDHA, SHCD and SDHD subunits being conserved.

Results: GISTs can be divided into two groups: type 1 (the majority) that occurs in adults, are spindle cell and are usually associated with a CKIT or PDGFR mutation; or type 2 that occur in children or young adults, with epithelioid morphology, CKIT/PDGFR WT and germline mutations in succinate dehydrogenase (SDH) subunits. Thus, type 2 GISTs are associated with a germline mutation of succinate dehydrogenase, which suggests they may be caused by defective mitochondrial oxidation; This is shown by the recent data that implicate this enzyme with this tumor as well as with others of a neuroendocrine nature. In addition, our patient had an associated history of abdominal paraganglioma, a dyad that has been identified as "Carney-Stratakis syndrome" (CCS). Carney-Stratakis syndrome occurs equally in both sexes and has autosomal dominant inheritance. It is a rare disease and is caused by germline mutations in succinate dehydrogenase B, C or D subunits, leading to tumor formation.

Conclusions: All these data should cause us to wake up, since they have clinical implications for patients with GIST that do not present the CKIT and PDGFRA (WT) mutation, since, on the one hand, these tumors are usually resistant to treatment with tyrosine inhibitors. kinase currently available, and on the other hand they and their relatives must be thoroughly studied because it may be a familial syndrome such as TC or SCC, especially if they are young patients.

[1] Who Classification of Tumours Editorial Board. (2020). Gastrointestinal Tumours: Who Classification of Tumours (5th ed.). World Health Organization. 45-47

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Development of an in vitro model of mesenteric ischemia/reperfusion injury

Montejo U.[1], Herrero de la Parte B.[2] and Alonso-Varona A.[1]

[1] Tissue Engineering Group, Department of Cell Biology and Histology, Faculty of Medicine and Nursing, University of the Basque Country, Biscay, Spain; [2] Department of Surgery, Radiology and Physical Medicine, Faculty of Medicine and Nursing, University of the Basque Country, Biscay, Spain

INTRODUCTION: Ischemia/reperfusion injury (IRI) is a serious consequence of mesenteric ischemia, which is caused by insufficient blood perfusion in the intestine. Although blood flow should be restored as fast as possible, reperfusion itself is also a major contributor to the final tissue damage. This is due to the generation of reactive oxygen species (ROS) and the subsequent oxidative stress and inflammation. Several studies have pointed out that the control of the sources of oxidative stress and inflammation during reperfusion are key to the prevention and treatment of IRI [1].

The aim of this work is to develop an *in vitro* model of mesenteric ischemia/reperfusion, based on oxygen and glucose deprivation and reperfusion (OGD/R). This model should serve as a platform for the study of the therapeutical efficacy of various IRI treatments.

MATERIALS AND METHODS: Rat intestine epithelial cells (IEC-6, ATCC collection) were cultured in DMEM supplemented with 10% FBS and insulin (0.1 U/mL), and seeded in 96 well plates (n=5). The culture medium was then replaced by Hanks' buffer (glucose deprivation medium) or complete DMEM medium as control. The plates were then incubated for 4 h at 37°C in a nitrogen-filled (oxygen deprivation) or normoxic (control) incubator. Cell viability and intracellular ROS levels were assessed using PrestoBlue™ and 2',7'-dichlorodihydrofluorescein diacetate (H2DCF-DA), respectively. To simulate reperfusion, the culture media were changed back to complete medium and the plates were incubated again for another 24 h in a normoxic incubator to restore oxygen and glucose. Finally, cell viability and intracellular ROS levels were analyzed again to study the effect of reperfusion.

RESULTS: Oxygen deprivation alone caused a loss of 59% of IEC-6 cell viability with respect to the control, while glucose deprived cultures showed less damage (13% loss). However, the combination of oxygen and glucose deprivation did not result in an increased damage over glucose deprivation alone. Oxygen and glucose deprivation also caused increased intracellular levels of ROS ($p<0,05$) independently, but the combination of both had no summatory effect on ROS. After reperfusion, the previously oxygen deprived cells still showed 56% less viability compared to the control group. The viability of the glucose deprived cells diminished further during reperfusion, showing 30% less viability than the control. The cultures subjected to oxygen and glucose deprivation still showed no difference in viability with respect to cells deprived of glucose alone. However, there was no statistically significant difference between the ROS levels of OGD/R and control cell cultures.

DISCUSSION & CONCLUSIONS: 4 h of oxygen and glucose deprivation followed by 24 h of reperfusion resulted in significant IEC-6 cell damage. These experimental conditions could serve as a basis for testing the therapeutic efficacy of mesenteric IRI treatments. However, the low levels of ROS measured after reperfusion contradict previous studies conducted with the same cell line and similar experimental procedure [2]. For this reason, we need to explore additional markers of oxidative stress, such SOD, MDA or GSH.

Supported by GIU19/088 (Tissue Engineering Group).

[1] Bertoni S, et al. Drug Discovery Today. 2018;23(7):1416-25
[2] Yang J & Xie X. Exp. Ther. Med. 2021;22:1108

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Ultrastructural Analysis of Philopodium Formation Induced by the SARS-CoV-2 Virus Infection in Vero E6 cells

Baselga M.[1,2], Uranga I.[2,3], Moreo E.[2,3], Arias M.[2,3], Monleón E.[1,2], Güemes A.[2,4], Pérez J.[1], Sarría S.J.[1] and Junquera C.[1,2]

[1] Department of Anatomy and Histology, University of Zaragoza, Zaragoza, Spain; [2] Instituto de Investigación Sanitaria Aragón (IIS Aragón), Zaragoza, Spain; [3] Department of Microbiology, Pediatrics, Radiology and Public Health, University of Zaragoza, Zaragoza, Spain, [4] Department of Surgery, University of Zaragoza, Zaragoza, Spain

Introduction: In cells, the cytoskeleton plays a crucial role in numerous viral processes, including cell entry, transport to cellular regions for viral replication and assembly, and release of new viruses. However, the alteration of the cytoskeleton after infection with SARS-CoV-2 in epithelial cells has been scarcely reported. This work aims to ultrastructurally characterize the formation of filopodia associated with the alteration of the cytoskeleton, such as its participation in cell-cell interaction.

Methods: Vero E6 cells were infected with the SARS-CoV-2 virus isolated from the parent variant and cultured for 48 hours. Conventional Electron Microscopy was performed.

Results: The formation of filopodia associated with SARS-CoV-2 infection was observed. The infected cells showed an evident development of the cytoskeleton, with characteristic and frequent formations of filopodial bridges between cells. Two different types of filopodial structures were found: on the one hand, interfilopodial connections without membrane fusion (referred to in this work as 'phyllopodial bridges') and with membrane fusion (referred to as 'tunneling nanotubes').

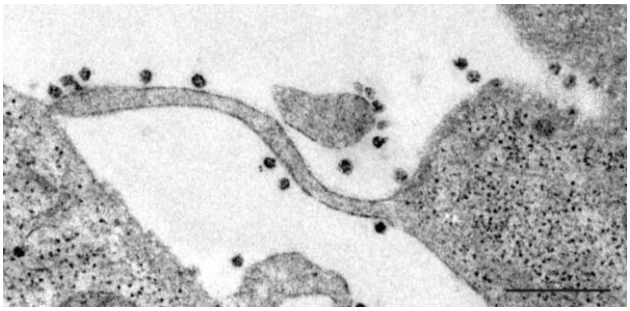


Fig 1. Viral surfing along a phyllopodial bridge.

Filopodial bridges and nanotubes could promote viral cell-cell propagation from two different phenomena: on the one hand, viral 'surfing', characterized by the transport of viruses through the surface of filopodia mediated by receptors and, on the other hand, the intracytoplasmic route, where viruses could travel from cell to cell inside the filopodia [1][2]. Even filopodia structures were found connecting more than two cells at a time. The structure of a filopodium was characterized by ordered bundles of F-actin and protein packaging of a few tens of nanometers. A higher density of viral particles has been identified in the membrane of the filopodia concerning other regions of the cell membrane, which could represent a preferential binding zone for the viruses; viral cell-cell propagation was frequently observed via the viral surfing route. In contrast, the intracytoplasmic route was occasionally identified so that viral surfing could represent the preferred transmission route for cell-cell infection.

Discussion & conclusions: The formation of numerous and extensive filopodia structures has been observed in virus-infected cells. These membrane projections have played a relevant role in probing the extracellular environment, directed migration, and cell-cell communication processes. The high concentration of viral particles attached to the filopodia observed in the electron micrographs in this work suggests that these membrane projections could be critical during viral infection and, therefore, could represent strategic and novel therapeutic targets.

This research was funded by the Instituto de Investigación Sanitaria Aragón: Campaña Investiga Covid-19. Authors would like to acknowledge the use of Servicio General de Apoyo a la Investigación (SAI), Universidad de Zaragoza.

[1] Caldas, L.A.; Carneiro, F.A.; Higa, L.M.; Monteiro, F.L.; da Silva, G.P.; da Costa, L.J.; Durigon, E.L.; Tanuri, A.; de Souza, W. Ultrastructural Analysis of SARS-CoV-2 Interactions with the Host Cell via High Resolution Scanning Electron Microscopy. *Sci. Rep.* **2020**, *10*, 1–8

[2] Chang, K.; Baginski, J.; Hassan, S.F.; Volin, M.; Shukla, D.; Tiwari, V. Filopodia and Viruses: An Analysis of Membrane Processes in Entry Mechanisms. *Front. Microbiol.* **2016**, *7*, 1–13

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Use of digital infrared thermal imaging for field cancerization detection of actinic keratosis

Shiguetomi-Sifuentes A.L.[1], Cruz-Ramos J.A.[1,2], Ramos-Márquez M.E.[1,2], López-Armas G.C.[3], De la Mora-Jiménez E.,[1] Peregrina-Barreto H.[4], Farías-López M.[1] and Falconi-Olán E.[1]

[1] Instituto Jalisciense de Cancerología, Guadalajara, Jalisco, México; [2] Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, México; [3] Centro de Enseñanza Técnica Industrial, Guadalajara, Jalisco, México; [4] Instituto Nacional de Astrofísica, Óptica y Electrónica, Tonantzintla, Puebla, México.

Introduction: Actinic keratosis (AK) is a chronic disease, affecting photoexposed areas, consisting of pre-malignant skin lesions due to chronic sun exposure and presents a high risk of progression to squamous cell carcinoma. Surgery do not always excise all potentially malignant cells. The areas visible under the dermatoscope do not detect the presence of multiple subclinical AK adjacent to a visible lesion called the field cancerization (FC), so it is difficult to perform a complete excisional biopsy that includes the AK lesion and its FC, which can lead to recurrence of the lesion. Therefore, methods such as digital thermography are suggested since it is non-invasive, easy to use and gives immediate results that shows hot spots in the digital infrared thermal image. (Fig. 1) Methods: Fifteen patients with initial clinical and dermatoscopic diagnosis of AK in the Antiguo Hospital Civil de Guadalajara "Fray Antonio Alcalde" were included. A biopsy was taken of the AK, as well as of the perilesional hyperthermic halo and healthy skin (HS). The histologic features evaluated in AK, FC and HS were: atypical parakeratosis, keratinocyte vacuolization, atypical basaloid keratinocytes, inflammatory infiltrate, nuclear pleomorphism, lack of maturation, mitotic figures, and hyperplasia. (Fig. 5) The thermographs were taken with a FLIR T600 camera and images obtained were analyzed with Image J software (Fig. 2). Statistic analysis was performed using SPSS software. A value of $p<0.05$ was taken as significant.

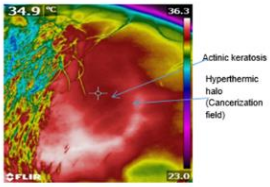


Fig. 1. Example of an image of actinic keratosis and field cancerization evaluated by thermography.

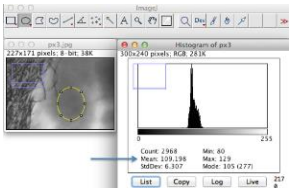


Fig. 2. Transformation of the thermographic image to gray spectrum to determine the intensity value.

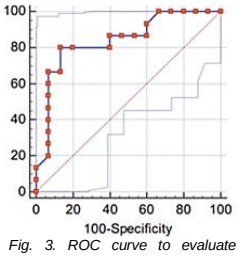


Fig. 3. ROC curve to evaluate accuracy of thermography to discriminate the lesion from healthy skin via monochrome intensity.

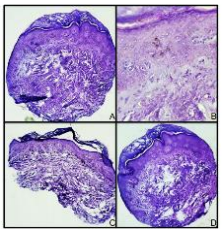


Fig. 4. Histopathological evaluation with Hematoxylin and Eosin. Histopathology of skin biopsy with changes associated with chronic sun exposure.

Results: A correlation was found between daily sun exposure and the amount of AK (Spearman correlation of 0.61, p= 0.016); a higher dermoscopic degree of AK was observed in photoexposed areas of the upper extremities, (p= 0.002 by Mann U). Hyperplasia correlated directly and atrophy inversely with the dermoscopic grade of AK (spearman's rho 0.675 and -0.682, respectively). In 100% of the AKs, a FC was documented, corresponding to a hyperthermic halo (difference of 0.3ºC SD +/- 0.2). There was a correlation between the temperature of the AK and the FC, with a Pearson coefficient of 0.998 and a value of p< 0.000. A Student's T test was performed for two independent samples between the means of the intensity values of AK and FC, with a value of p= 0.03. A ROC curve was performed to differentiate FC from healthy perilesional skin, obtaining a cut-off point of 100, based on pixel intensity (sensitivity of 80% and specificity of 86%, p=0.0001). (Fig. 4) Discussion & conclusions: Digital infrared thermography is a non-invasive and objective diagnostic method to assess the FC in AK. The analysis of intensity by thermography is useful as a diagnostic method to discriminate AK, FC and HS.

[1] Siegel JA., Korgavkar K. and Weinstock MA. (2017). Current perspective on actinic keratosis: a review Br J Dermatol. 177(2):350-358.
[2] Baek YS., Kim J., Song JY., Jeon J. and Oh CH. (2019). Dynamic thermal imaging on actinic keratosis patients: A preliminary study. Skin Res Technol. 25(2):211-216
[3] Laino L., Elia F., Desiderio F., Scarabello A., Sperduti I., Cota C. and DiCarlo A. (2015). The efficacy of a photolyase-based device on the cancerization field: a clinical and thermographic study. J Exp Clin Cancer Res. 19;34(1):84.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Gestational venous hypertension produces histopathological changes in the human placenta and in the umbilical cord with implications in maternal-fetal welfare

Fraile-Martinez O.[1,2*], Ortega M.A.[1,2], Garcia-Montero C.[1,2], Guijarro L.G.[2,3], Saez M.A.[1,2,4], Ruiz-Grande F.[5], Monserrat J.[1,2], Alvarez-Mon M.[1,2,6], García-Honduvilla N.[1,2] and Bujan J.[1,2]

1Universidad de Alcalá, Department of Medicine and Medical Specialties, Faculty of Medicine and Health Sciences, 28801 Alcalá de Henares, Spain. 2Ramón y Cajal Institute for Health Research (IRYCIS), 28034 Madrid, Spain. 3University of Alcalá, Unit of Biochemistry and Molecular Biology, Department of Systems Biology (CIBEREHD), 28801 Alcalá de Henares, Spain 4Service of Pathological Anatomy, Hospital Universitario Central de la Defensa-UAH, 28001 Madrid, Spain. 5Vascular Surgery Department, Hospital de la Princesa, 28834 Madrid, Spain. 6Department of Internal Medicine and Diseases of the Immune System-Rheumatology, Oncology (CIBEREHD), Hospital Universitario Príncipe de Asturias, 28806 Alcalá de Henares, Spain. *Correspondence author: oscarfra.7@hotmail.com

Gestational venous hypertension (HVG) is an entity caused by common hyperpressure from the third trimester. This fact was due to an increase in pressure in the pelvic region due to an increase in the tissue structures and the growth of the fetus. We conducted an observational, analytical, prospective study nested in a cohort with a total of 62 patients diagnosed with HVG from the third trimester, compared with 52 women without HVG. We have performed a complete histopathological study of the placenta and umbilical cord, in addition to performing immunohistochemical and RT-qPCR techniques. Our results show how the placenta of women diagnosed with HVG show histopathological changes compatible with tissue damage, together with an increase in hypoxic damage proteins, remodeling and oxidative stress. These data are shown in parallel in the umbilical cord in parallel, with an association with data on loss of fetal well-being.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Carbon–based Nanoparticles for Sentinel Node Labeling: Ultrastructural Study

Baselga M.[1,2], Monleón E.[1,2], Güemes A.[2,3], Arribas M.D.[2,3], Arruebo M.[2,4,5], Sebastián V.[2,4,5] and Junquera C.[1,2]

[1] Department of Anatomy and Histology, University of Zaragoza, Zaragoza, Spain; [2] Instituto de Investigación Sanitaria Aragón (IIS Aragón), Zaragoza, Spain; [3] Department of Surgery, University of Zaragoza, Zaragoza, Spain; [4] Department of Chemical and Environmental Engineering, University of Zaragoza, Zaragoza, Spain; [5] Instituto de Nanociencia y Materiales de Aragón (INMA), CSIC-Universidad de Zaragoza, Zaragoza, Spain

Introduction: Given the limitations presented by current techniques used to tag the sentinel lymph node (SLN) in breast cancer patients who have undergone neoadjuvant therapy, the aim is to carry out a tag based on nanoparticles to be able to identify the treated node after treatment. Those nanoparticles must be durable, stable in the node without perceptible uncontrolled migration, and biocompatible. There is no previous ultrastructural study that analyzes the interaction of tagging nanoparticles with the different cell types that make up the sentinel node. (The Histological study is presented in another Communication).

Methods: As a tagging pigment, poly(lactic-co-glycolic acid)–based (PLGA) particles having encapsulated carbon nanoparticles (C-NPs) in their interior have been developed. The particles have been synthesized using two different techniques: on the one hand, simple emulsion-solvent evaporation and, on the other hand, electrohydrodynamic techniques. The nanoparticles obtained by emulsion showed submicrometric diameters (100-150 nm), while the microparticles resulting from electrospinning reached larger sizes (1-2 μm). However, the polymer-carbon ratio was similar (88 ± 3 wt.% PLGA and 12 ± 2 wt.% C-NPs). The obtained particles were suspended in a viscous carrier and injected by direct puncture into pig lymph nodes. The animals were kept under standard conditions for at least 6 weeks. Once the biopsies of the tagged lymph nodes were obtained, they were routinely processed to observe them using a Transmission Electron Microscope (TEM). The proper care and handling of the animals was included in a protocol authorized by the Animal Experimentation Commission of Aragon (PI09/20) according the Directive 2010/63/EU.

Results: Nanoparticle-based tags persisted in the evaluated nodes for up to 6 weeks. Free carbon-based nanoparticles (used as controls) and the micro and nanoparticles were not endocytosed by any of the cell types that constitute the cellular stroma of the lymph node (lymphoblasts, B and T lymphocytes, dendritic cells, and monocytes), but, conversely, they were phagocytosed by macrophages. No nanoparticles were observed inside lymphatic vessels. The macrophages phagosomes presented differences in size after particle internalization, and the type of endocytosed particle could be perfectly distinguished inside. In all three particles studied, the carbon-based nanomaterial causes the rupture of the phagolysosome membrane, spilling the lysosomal hydrolases into the cytosol, which leads to the lysis of the cell membrane with the spillage of the cellular organelles into the extracellular space. New macrophages are responsible for phagocytizing cell debris. A foreign body reaction also occured, and multinucleated giant cells isolating the pigment were observed in some cases.

Discussion & conclusions: The tagging composed of free no-encapsulated carbon nanoparticles produces an immediate inflammatory reaction, while the use of micro and nanoparticles as encapsulating matrices for the carbon-based nanoparticles manages to lengthen the residence time inside the phagosomes. Together with the histological study, we can conclude that the macrophages drag the pigment towards the lymph node capsule, where the pigment remains stable over time, providing a positive identification of the sentinel node.

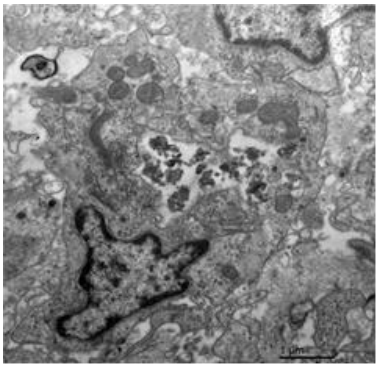


Fig 1. Phagosomes with carbon in a macrophage

Supported by Instituto de Salud Carlos III (ISCIII) project DTS20/00131. Authors would like to acknowledge the use of Servicio General de Apoyo a la Investigación-SAI, Universidad de Zaragoza

[1] Najahi-Missaoui W. et al. Int. J. Mol. Sci. 2021, 22, 385
[2] Jiang Y, et al. Front Oncol. 2022;12:818812

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Gastrointestinal cancer: Relationship between histology and microbiota

García Sanz M.[1], Escamilla Sánchez A.[1], Mercado-Sáenz S.[1], Rodríguez Pérez L.M.[1], Arranz Salas I.[1,2], Sánchez Varo R.[1], Peláez González A.[1], López Villodres J.A.[1] and Bermúdez Flores D.[1]

[1] Medical Biology and Histology PAI Reference: CTS429. Department of Human Physiology, Human Histology, Pathological Anatomy and Physical Sport Education. School of Medicine. Malaga University. Spain; [2] Unit of Anatomical Pathology, Virgen de la Victoria University Hospital, 29010 Malaga, Spain

Objectives: Review of the published literature concerning the relationship between microbiome and gastrointestinal cancer.
Methods: Present work is focused on systematic research in the most prominent biomedical databases finds relevant works in Pubmed and the library's catalog of the University of Málaga (Jábega) of published journals in the last 5 years.
Results: In this work, the mechanisms used by the microbiome to damage gastrointestinal epithelial cells and cause cancer are explained. Some of them are the dysbiosis, destruction of the mucosal barrier, chronic inflammation, damage caused by metabolites produced in the digestion and the direct attack of certain toxins to the cell's DNA. These mechanisms adjust the immune response, by activation or inhibition using different cytokines. There is also a deeper look into several microorganisms and how they cause gastrointestinal cancer using toxins or virulence factors to activate them.
Conclusions: The evidence found so far about the microbiota and gastrointestinal cancer is enough to assume the relationship between them, although there is much left to research. With these findings, it can be expected that in a near future certain microorganisms could be used for screening purposes, due to their increase in early stages of the tumor genesis and also, in a preventive way to try to eradicate them, even avoid cancer. Studies on the microbiota are hardly beginning, and results appear to be promising.

Keywords: gut + microbiome + cancer + precancerous + histology + microbiota

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Carbon and melanin based nanoparticles for lymph node labeling

Monleón E.[1,2], Baselga M.[2], Junquera C.[1,2] Mendoza G.[2], Yus C.[2,3,4], Alejo T.[2,3,4] and Güemes A.[2,5]

[1] Department of Anatomy and Histology, University of Zaragoza, Zaragoza, Spain; [2] Instituto de Investigación Sanitaria Aragón (IIS Aragón), Zaragoza, Spain; [3] Department of Chemical and Environmental Engineering, University of Zaragoza, Zaragoza, Spain; [4] Instituto de Nanociencia y Materiales de Aragón (INMA), CSIC-Universidad de Zaragoza, Zaragoza, Spain. [5] Department of Surgery, University of Zaragoza, Zaragoza, Spain

Introduction: In breast cancer patients, labeling of the sentinel lymph node is used to facilitate its relocation at the time of biopsy after neoadjuvant therapy. There are numerous types of labels such as metallic clips, radioactive seeds or endomagnetic markers. These techniques have some drawbacks as displacement of the clips, the use of radiation or the need to access certain materials and equipment [1]. The aim of this study was to assess the lymph node marking intensity and the histological changes induced after injection of two types of labels based on carbon and melanin nanoparticles (NPs).

Methods: As labels, poly (lactic-co-glycolic acid)–based (PLGA) particles having encapsulated carbon nanoparticles or melanin were synthesized using the simple or double emulsion-solvent evaporation techniques respectively. Similar nanoparticles size and morphology were obtained using both compounds: 123 ±32 nm in diameter for carbon and 117 ±38 for melanin. Carbon based NPs development is described in more detail in Baselga et al. communication (Carbon–based Nanoparticles for Sentinel Node Labeling: Ultrastructural Study).

The two types of labels were inoculated into 2 or 3 mesenteric lymph nodes from 4 healthy female pigs. Animals were sacrificed at 1, 2, 3 and 6 weeks after inoculation and the mesenteric lymph nodes were collected and fixed in 10% formalin. Procedures were approved by the Animal Experimentation Ethical Commission, University of Zaragoza (permit number: PI09/20). Macroscopically, the marking intensity was subjectively scored based on the extent of labeling. After, formalin-fixed tissues were trimmed and processed according to standard histopathological procedures. Microscopically, location and morphology of the pigments and histological changes associated to them were evaluated.

Results: Carbon based NPs showed a high labeling intensity that was maintained throughout the study period. In the case of melanin based NPs, the intensity was high 1 week after inoculation but gradually decreased, although in all samples labeling was macroscopically visible. In both types of labeling, the pigments were located pericapsular or / and in the soft tissue surrounding the node. Microscopically, melanin based NPs presented a quite homogeneous morphology characterized by small granules of pigment. In C-NPs samples, abundant small granules combined with deposition of coarse clumps of pigment were observed. All samples showed a foreign body reaction associated with the pigments. Foci of necrosis associated with foreign body reaction were observed in melanin based NPs 1 week after inoculation and in carbon based NPs 6 weeks after inoculation.

Discussion & conclusions: The carbon and melanin based NPs shown in the present study may be an alternative approach to label lymph nodes as they can be identified macroscopically and remain in place for at least 6 weeks. These labels are relatively inexpensive, require no special equipment, and reduce the disadvantages of other techniques mentioned above. However, although carbon is believed to be nontoxic [2], we show that both carbon and melanin based NPs induce a foreign body reaction evident 1 week after injection and maintained until week 6. In the case of melanin based NPs, the tissue response was higher initially and progressively decreased, while carbon based NPs showed a lower inflammatory reaction after inoculation that progressively increased in intensity. More histological and clinical studies are needed to establish the significance of this tissue reaction.

Supported by Instituto de Salud Carlos III (ISCIII) project DTS20/00131. Authors would like to acknowledge the use of Servicio General de Apoyo a la Investigación-SAI, Universidad de Zaragoza.

[1] Zhang L, et al. World J Surg Oncol. 2018 Jun 19;16(1):112.
[2] Xie P, et al. Int J Mol Sci. 2017 Nov 29;18(12):2562.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Nanoparticles of maqui polyphenolic extract for the treatment of inflammatory bowel disease

García-Esteban I.[1;2], Ortiz T.[1], Merinero M.[1], García-García MD.[1], Zaderenko A.P.[2], Macías-García L.[1], Vázquez-Román V.[1] and De-Miguel M.[1]

[1] Department of Normal and Pathological Cytology and Histology. School of Medicine. University of Seville, 41009 Seville, Spain; [2] Area of Physical Chemistry. Department of Physical, Chemical and Natural Systems. Faculty of Experimental Sciences. University of Pablo de Olavide, 41013 Seville, Spain.

Introduction: Inflammatory bowel disease (IBD) is a digestive tract disorder, of which two major types are distinguished: ulcerative colitis (UC) and Crohn's disease (CD). Although the etiology of IBD is unknown, it is known to be a multifactorial disease. The prevalence and incidence of UC and CD have increased worldwide in recent decades [1, 2]. Polyphenols, and among them those from the tannic acid and maqui extract, are showing great potential in the treatment of IBD, thanks to its anti-inflamatory and antioxidant properties [3]. It remains a challenge to find a formulation that allows them to reach their site of action, so we have developed formulations based on polymeric organic nanoparticles containing these polyphenolic extracts.
Methods: We have encapsulated maqui polyphenolic extract in polymeric organic nanoparticles based on a co-precipitation method developed in our group [4]. On the other hand, these nanoparticles have been administered to a murine acute model of CD as treatment. The disease was induced with TNBS at a concentration of 100 mg/kg with 50% EtOH and the treatment lasted the 4 days after. At day of sacrifice, the large intestine was removed, cut longitudinally in two and rolled from the distal end to the proximal end forming a "roll" for subsequent histopathological and immunohistochemical study. This was fixed in formaldehyde and embedded in paraffin. Serial sections were made and stained with H&E and others were stained using the immunohistochemical technique.
Results: The histopathological study showed the area of the colonic tissue intact in most of its extension, presenting preservation of all its layers and slight acute and chronic inflammatory infiltrate and loss of goblet cells, while in the samples analyzed from the control group it is observed that the colonic tissue is intact and mild inflammatory infiltrate. However, the immunohistochemical study for the control group showed weak staining for the NLRP6 inflammasome. Immunostaining was negative for goblet cells and the enterocytes only expressed a slight cytoplasmic staining, reinforced in the cells at the base of the intestinal glands and weak at the superficial level. While for the group treated with maqui nanoparticles, it did not become negative, but it was very weak in much of the mucosa, expressing weakly in the cells of the intestinal crypts.
Discussion & conclusions: These results suggest that the nanoparticles administered in those animals with treatment, showed a greater recovery in histological damage in the colon. On the other hand, they presented a protective activity on inflammation since the colonic tissue remained intact without glandular loss in most of its extension. In the immunohistochemical analysis of the NLRP6 inflammasome, it is observed that the treated group showed a significant reduction, matching the control group. Therefore, this study shows a possible effective therapy against CD thanks to the beneficial properties of polyphenolic extracts, and it would be necessary to continue investigating to go one step further and carry out a clinical trial with patients.

[1] Yu, Y. R., and Rodriguez, J. R. (2017). Clinical presentation of Crohn's, ulcerative colitis, and indeterminate colitis: Symptoms, extraintestinal manifestations, and disease phenotypes. *Seminars in Pediatric Surgery*, *26*(6), 349-355
[2] Cosnes, J., Gowerrousseau, C., Seksik, P., and Cortot, A. (2011). Epidemiology and natural history of inflammatory bowel diseases. *Gastroenterology*, *140*(6), 1785-1794
[3] Masoodi, H., Villaño, D., and Zafrilla, P. (2019). A comprehensive review on fruit: Aristotelia chilensis (Maqui) for modern health: Towards a better understanding. *Food and Function*, *10*(6), 3057-3067
[4] Aguilera, J. R., Venegas, V., Oliva, J. M., Sayagués, M. J., De Miguel, M., Sánchez-Alcázar, J. A., and Zaderenko, A. P. (2016). Targeted multifunctional tannic acid nanoparticles. *RSC Advances*, *6*(9), 7279-7287

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Randomized clinical study in three types of sutures in oral surgery. Tissue reaction, bacterial colonization and clinical characteristics

Sánchez-Porras D.[1,2], Morales Bancalero S.[3], Arias-Moliz M.T.[4] and Romero-Olid M.N.[5]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Spain, Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain, [3] Graduated in Dentistry. University of Granada, [4] Prof. Head of Microbiology. Department of Microbiology, University of Granada, Spain, [5] Prof. Associate of Oral Surgery and Implantology, Department of Stomatology, University of Granada, Spain

Introduction: Surgical site infections (SSIs) represent an important cause of morbidity and mortality in patients undergoing surgical interventions, with a prevalence in Spain of 11%. The appearance of these infections has been associated with a wide variety of factors, among which the contamination of the suture materials due to the presence of microorganisms stands out. Being suture a vital part of all surgical procedures, its choice can influence wound healing, especially in the oral cavity, due to humidity conditions, presence of saliva and microorganisms and continuous food intake, constituting an ideal substrate for microbial adhesion and subsequent biofilm formation. In this study, three surgical sutures (Silk®, PTFE® and Daclon®) are compared in relation to tissue reaction, bacterial colonization, and clinical characteristics of each of them.

Methods: Five patients who underwent extraction of an impacted lower third molar using a bayonet incision approach were selected. Said incision was sutured using the 3 study materials that were randomly placed using a computer-generated random number table, with the aim of placing two equal sutures in the horizontal incision (position 1), another suture in the upper point of the discharge (position 2) and the last one at the lower point of the discharge (position 3). The sutures were removed 7 days after the intervention.

A histological study with hematoxylin-eosin was performed on the parts of the suture that remained inside the wound to analyze the inflammatory reaction. Likewise, in vitro biocompatibility was evaluated using the WST-1 technique (Roche Applied Science, Penzberg, Germany) in cultures of human fibroblasts derived from oral mucosa at 24, 48 and 72 hours in direct contact with the sutures. For the microbiological study, the part of the suture that was exposed to the oral environment was used. The level of bacterial contamination was determined by counting viable cells. In addition, the clinical parameters looseness, manageability, healing, pain and inflammation were studied from the day of surgery to the day of suture removal.

Results: The histological results highlight that the greatest inflammatory reaction occurred with silk (Silk®), followed by PTFE® and finally Daclon®. The viability of metabolically active fibroblasts was similar in the three types of sutures. However, with respect to the positive control, there is a decrease in cellular activity in the three sutures. No statistically significant differences were observed between the sutures in bacterial contamination. In the clinical parameters studied, looseness did not show significant differences, manageability was higher in silk (Silk®), the degree of healing was similar between patients, and pain and inflammation decreased from the first to the seventh day. In microbiological analysis, although there were no statistically significant differences in viable cell count among the three sutures, silk (Silk®) showed higher bacterial contamination.

Discussion & conclusions: Healing was quite favorable. Pain and inflammation followed a decreasing trend. According to the histological and microbiological results, the physical configuration of the sutures plays a very important role in microbial adhesion and inflammatory reaction, which in both cases was greater with silk (multifilament) than with the other two sutures (monofilament). In conclusion, silk (Silk®) was the one that showed a greater inflammatory reaction and bacterial contamination. The looseness was similar in the three sutures, while the manageability was greater in silk and PTFE®, being even more superior in silk. Healing was quite good in all patients overall, and both pain and clinical swelling decreased over time.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

The morphology of the extracellular matrix as a classifier of types and subtypes of Ewing and Ewing-like sarcomas: A morpho-molecular study

López-Carrasco A.[1,2], Diaz-Martin J.[1,3], Machado I.[1,4], Navarro S.[1,2], de Alava E.[1,3] and Noguera R.[1,2]

[1] Cancer CIBER (CIBERONC), Madrid, Spain. [2] Department of Pathology, School of Medical, University of Valencia-INCLIVA, Valencia, Spain. [3] Institute of Biomedicine of Sevilla, Virgen del Rocio University Hospital/CSIC/University of Sevilla/CIBERONC, Seville, Spain. [4] Pathology Department, Instituto Valenciano de Oncología, Valencia, Spain.

Introduction: Round cell sarcomas are a heterogeneous group of tumors that often affect children and young adults and can follow a very aggressive clinical course. There exist specific subtypes of round cell sarcoma, such as Ewing sarcoma (ES) which respond to well-defined therapeutic regimens, so the proper triage is crucial for an accurate patient management. A subset of the round cell sarcomas, however, lack clinical, morphologic, and specific immunophenotypic markers, and cannot be unequivocally classified on the basis of such features. In the last years a small number of these atypical cases with undifferentiated appearance, called generically "ES-like" have been characterized as carriers of BCOR-CCNB3 or CIC-DUX4 or EWSR1-NFATc2 fusions [1], but its morphological features and the properties of its extracellular matrix (ECM) remain unstudied. Reticulin fibers (RT fibers), main elements of tissue scaffolding, glycosaminoglycans (GAGs), with the ability to retain water, and vitronectin (VN), for anchoring cells to the ECM, seem to play an important role in the aggressiveness of some tumors [2]. Characterizing these elements in ES and especially in ES-like could help to improve the classification and prognostic of these tumors and also to link ECM and genetic properties.

Methods: Samples from 4 cases of BCOR-CCNB3 sarcoma, 3 cases of CIC-DUX4 sarcoma, 1 case of EWSR1-NFATc2 sarcoma and 17 cases with typical EWS-Fli1 fusion (3 of them with atypical histology) were included in this study. Sections of tumor tissue microarrays containing the selected cases were stained with alcian blue to study the GAGs of the tumors and with Gomori's to analyze the content and morphology of RT fibers. Immunostainings with anti-VN were done to study the VN of the sample. Stained slides were subjectively evaluated under a microscope by a pathologist. Subsequently, the slides were scanned with the Ventana iscan HT (Roche). GAGs and VN were objectively evaluated by digital analysis using specifically designed algorithms for the QuPathTH software. RT fibers were evaluated using GomoriPath software.

Results: Subjective and digital analysis of the mentioned ECM elements showed that CICex20-DUX4ex1 sarcomas presented the highest amount of VN. Moreover, VN is especially low in EWSR1ex8-NFATC2ex3 sarcomas. The quantity of GAGs was found to be higher in the EWSR1ex8-NFATC2ex3 tumor than in the other ES-like. A typical ES with spindle cells showed a larger presence of GAGs compared to ES with conventional histology. Finally, we detected differences in some parameters that affect RT fibers such as their size and shape in ES in comparison to ES-like cases.

Discussion & conclusions: These results suggest that VN and GAGs could be interesting biomarkers in the classification of subtypes of ES-like. Moreover, RT fibers have different features between typical ES and ES-like. However, a greater number of tumors are needed to confirm these findings.

Supported by Cris Contra el Cáncer.

[1] Watson, et al. J Pathol 2018, 245: 29-40
[2] López-Carrasco, et al. J Exp Clin Cancer Res. 2020; 39: 226

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Internalized capillaries in regenerating muscles

Ruz-Caracuel I.[1,2,3], Peix-Raya M.[2], Peña-Toledo M.A.[1,4,5], López-Espejo M.E.[1,2], Gil-Belmonte M.J.[1,2,6], Leiva-Cepas F.[1,2,5,7] and Peña-Amaro J.[1,3]

[1]Muscle Regeneration Group, University of Cordoba, Spain; [2]Department Morphological Sciences. Section of Histology, Faculty of Medicine and Nursing, University of Córdoba, Spain; [3]Department of Pathology, Ramon y Cajal University Hospital, IRICYS, Madrid, Spain; [4]Dementia and Multiple Sclerosis Unit, Neurology Service, Reina Sofia University Hospital, Cordoba, Spain; [5]Maimonides Institute for Biomedical Research IMIBIC, Reina Sofia University Hospital; [6]Department of Pathology, Torrecardenas Hospital, Almeria, Spain; [7]Department of Pathology, Reina Sofia University Hospital, Cordoba, Spain.

Background: Skeletal muscle is a highly vascularized organ given its high metabolic demand. Histologically, capillaries are placed at the periphery of muscle fibers. However, some reports have described internalized capillaries; that are sometimes associated with splitting fibers. The main objective is to analyse if abnormal muscle regeneration favours internalized capillaries emergence. Methods: Skeletal muscle biopsies from human biopsies and two murine models were analyzed. One model corresponds to a normal regenerative process caused by a myotoxic agent; the other was a fibrotic process caused by a volumetric muscle loss. Human biopsies from two conditions were selected: inflammatory myopathies and muscles suffering from denervation. In both experimental and human muscle biopsies the following parameters were analysed: number of fibers with internalized capillaries, number of splitting fibers and the number or percentage of fibers with internalized nuclei. Results: An internalized capillary was observed in one biopsy from an inflammatory myopathy. In contrast, no internalized capillaries were observed in any of the experimental models. There were statistically significant differences in number of splitting fibers and the number and the percentage of fibers with internalized nuclei between the two experimental conditions. Discussion and conclusion: Based on our observations, internalized capillaries are not associated with the causative muscle lesion or the abnormal regeneration. This infrequent phenomenon must be associated with other causes.

Acknowledgments: Supported by CTS-985 Muscle Regeneration Group.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Magnetic stimulation activates satellite cells and reduces muscle lesions in experimental autoimmune encephalomyelitis: ultrastructural observations

Peña-Toledo M.A.[1,2,3], Ruz-Caracuel I.[1,4,5], Luque E.[3,4], Latorre M.[3,6], Leiva-Cepas F.[1,3,4,7], Jimena I.[1,3,4], Agüera E.[2,3], Peña-Amaro J.[1,3,4] and Túnez I.[3,4]

[1]Muscle Regeneration Group, University of Cordoba, Cordoba, Spain; [2]Dementia and Multiple Sclerosis Unit, Neurology Service, Reina Sofia University Hospital, Cordoba, Spain; [3]Maimonides Institute for Biomedical Research IMIBIC, Reina Sofia University Hospital; University of Cordoba, Cordoba, Spain.[4]Department Morphological Sciences. Section of Histology, Faculty of Medicine and Nursing, University of Córdoba, Spain; [5]Department of Pathology, Ramon y Cajal University Hospital, IRICYS, Madrid, Spain; [6]Department Biochemistry and Molecular Biology, Faculty of Medicine and Nursing, University of Córdoba, Spain; [7]Department of Pathology, Reina Sofia University Hospital, Cordoba, Spain.

Background: Experimental autoimmune encephalomyelitis (EAE), an experimental model of multiple sclerosis, causes muscle atrophy and neurogenic lesions in muscle fibers that can be reversed after treatment with magnetic stimulation (MS). Satellite cells are responsible for the phenomena of growth and regeneration of skeletal muscle and, consequently, play an essential role in the response to any therapeutic strategy against neuromuscular disorders. In this study we have investigated the effect of magnetic stimulation on the ultrastructure of muscle fibers and satellite cells in rats with EAE.

Methods: We used 28 Dark Agouti rats that were divided into four groups: i) Control group of normal rats, ii) Vehicle group of rats injected with complete Freund's adjuvant, iii) EAE group of rats immunized with myelin oligodendrocyte glycoprotein (MOG) and iv) EAE+MS Group. In this group the rats were treated with MS (60 Hz and 0.7 mT) for 2 h in the morning, once a day, 5 days a week, for 3 weeks (starting on day 15 post-immunization). The rats were sacrificed on day 36 after immunization and the soleus muscles were extracted and processed for evaluation by light microscopy with histological and immunohistochemical techniques and by transmission electron microscopy. Changes at the ultrastructural level were assessed semiquantitatively.

Results: Muscles in the EAE group had muscle fibers positive for acid phosphatase, fibers with core-targetoid lesions and others with occasional peripheral desmin+ protrusions. Semiquantitative electron microscopy analysis showed ultrastructural changes in muscle fibers and satellite cells. MS reduced acid phosphatase activity in muscle fibers and core-targetoid lesions and increased desmin+ profiles; ultrastructurally reduced abnormalities in muscle fibers and caused activation of satellite cells.

Discussion and conclusions: In conclusion, the attenuation of ultrastructural alterations in muscle fibers caused by EAE and the activation response of the satellite cell would be indicative of a plastic response of skeletal muscle to MS in EAE model.

Acknowledgments: Supported by CTS-985 Muscle Regeneration Group and GC28 Neuroplasticity and Oxidative Stress Group.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Are serum calcium and phosphorus levels indicative of the state of the bone trabeculae in patients with hip fracture?

Crespo P.V.[1,2], Cano J.R.[3], García J.M.[1,2], Crespo-Lora V.[4], Blanco-Elices C.[1,2], Garzón I.[1,2], Rodríguez I.A.[1,5], Campos A.[1,2], Cruz E.[3] and Guerado E.[3]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Spain; [3] Division of traumatology and orthopedic surgery, Hospital Costa del Sol, Marbella, Spain; [4] Unidad Provincial Intercentros Granada Anatomía Patológica. Granada, Spain; [5] Cátedra "B" de Histología y Embriología, Facultad de Odontología, Universidad Nacional de Córdoba, República Argentina

INTRODUCTION: Hip fractures in the elderly are a major public health problem, and the main risk factor associated to this fracture in old patients is traditionally considered to be traumatic rather than osteoporosis [1,2]. The objective of this work is to assess calcium and phosphorus levels in patients' serum with the state of the bone trabecula in patients with hip fractures in order to assess this parameter as a possible risk factor in these patients.
MATERIALS AND METHODS: Forty patients subjected to surgical arthroplasty were studied: 20 with femoral neck fracture and 20 with coxarthrosis. All patients received the same diagnostic protocol, and the same antibiotic, anesthetic, surgical and antithrombotic prophylaxis. Blood samples were taken from each patient to perform biochemical tests for serum calcium and phosphorus. For histological study, bone tissue samples were taken from the femur head at the moment of the surgery. Tissues were fixed in 4% buffered formaldehyde and demineralized for 7 days in EDTA, until the total disappearance of hydroxyapatite. After washing, the samples were embedded in paraffin, and 5 µm-thickness sections were obtained and stained with hematoxylin and eosin. Three different images of each sample were taken at 4 magnifications, which were processed using ImageJ 1.51w software connected to a Nikon Eclipse i90 optical microscope.
RESULTS: Blood levels of Calcium ($p=0.03$) and Phosphorous ($p<0.01$) were significantly decreased in the group of patients with hip fractures. However, no statistical differences were found regarding the histological structure of the bone trabeculae, and the number, area, width and intertrabecular distance were similar in both study groups. In contrast, statistically significant differences were found for the length of the trabeculae, which were higher in the group of patients with coxarthrosis ($p=0.002$), although differences were not significant between both genders. Analysis of patients of different age revealed that the intertrabecular distance was higher in patients older than 75 years ($p=0.036$), with no differences for the rest of parameters.
DISCUSSION & CONCLUSIONS: In this work, we found that the blood levels of Calcium and Phosphorous were significantly decreased in the study group. However, this decrease was not correlated with the quality of the bone according to previous works using analytic electron microscopy [1,2]. Histologically, we found differences between the length of the trabeculae and the intertrabecular distance, which increases with age. Therefore, we could conclude that treatment of osteoporosis should be focused on increasing the synthesis of bone material and on improving the microarchitecture of the bone trabeculae in order to strengthen the structure of the hip bone.

This study was supported by CTS-115 (Tissue Engineering Group).

[1] Cano JR, Crespo PV, Cruz E, Rivas-Ruiz F, Sánchez-Quevedo MC, Guerado E, Campos A. Is the bone tissue of the femoral neck demineralised in patients with hip fracture? Injury. 2020;51 Suppl 1:S4-S11
[2] Guerado E, Cruz E, Cano JR, Crespo PV, Alaminos M, Sánchez-Quevedo Mdel C, Campos A. Bone mineral density aspects in the femoral neck of hip fracture patients. Injury. 2016 Jan;47 Suppl 1:S21-4

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Antibacterial biopolymer gel coating on meshes used for abdominal hernia repair promotes effective wound repair in the presence of infection

Benito-Martínez S.[1,2,3], Pérez-Köhler B.[1,2,3], Rodríguez M.[2,3,4], García-Moreno F.[2,3,4], Gómez-Gil V.[2,4,5], Bellón J.M. [2,3,4] and Pascual G.[1,2,3]

[1] Departamento de Medicina y Especialidades Médicas, Facultad de Medicina y Ciencias de la Salud, Universidad de Alcalá, Madrid, Spain; [2] Biomedical Networking Research Centre on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN, ISCIII), Madrid, Spain; [3] Ramón y Cajal Health Research Institute (IRYCIS), Madrid, Spain; [4] Departamento de Cirugía, Ciencias Médicas y Sociales, Facultad de Medicina y Ciencias de la Salud, Universidad de Alcalá, Madrid, Spain; [5] Departamento de Ciencias Biomédicas, Facultad de Medicina y Ciencias de la Salud, Universidad de Alcalá, Madrid, Spain.

Background: Prosthetic mesh infection is a devastating complication of abdominal hernia repair which impairs natural healing in the implant area, leading to increased rates of patient morbidity, mortality, and prolonged hospitalization. This preclinical study was designed to assess the effects on abdominal wall tissue repair of coating meshes with a chlorhexidine- or rifampicin-loaded carboxymethylcellulose biopolymer gel in a *Staphylococcus aureus* (*S. aureus*) infection model.
Methods: Partial hernia defects (5 x 2 cm) were created in the right anterior side of abdominal wall in New Zealand white rabbits (n = 20). Four study groups (n= 5 each) were established according to whether the meshes were coated or not with each of the antibacterial gels. Three of the groups were inoculated with *S. aureus* and subsequently repaired with a lightweight polypropylene mesh (Optilene Mesh Elastic), either uncoated or coated with the corresponding gel. The last group was not infected and received a bare mesh, as control. Fourteen days after surgery, implanted meshes were recovered for histological studies, analysis of the gene and protein expression of collagens, macrophage phenotypes (M1 and M2), and mRNA expression of vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs). Data were expressed as the mean ± standard error. To compare different study groups, the Mann-Whitney U test was used. All statistical tests were performed using GraphPad Prism 5 software.
Results: Compared to uncoated ones, antibacterial-coated meshes showed good integration within the host tissue, as well as higher collagen 1/3 mRNA ratio and collagen I protein expression, relatively increased VEGF mRNA expression, a significantly reduced macrophage response, and lower relative amounts of MMPs mRNAs.
Discussion and conclusions: Our findings suggest that prophylactic antibacterial coating of meshes may help improving abdominal wall tissue repair in the presence of infection.

Supported by Grants SAF2017-89481-P and PDC2021-121809-I00 from the Spanish Ministry of Science and Innovation, and CIBER-BBN.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Biological and mechanical evaluation of mesh fixing devices for hernia repair: self-gripping/self-adhering vs synthetic adhesives

Pérez-Köhler B.[1,2,3], Benito-Martínez S.[1,2,3], Rodríguez M.[2,3,4], García-Moreno F.[2,3,4], Calvo B.[5], Peña E.[5], Bellón J.M. [2,3,4] and Pascual G.[1,2,3]

[1] Departamento de Medicina y Especialidades Médicas, Facultad de Medicina y Ciencias de la Salud, Universidad de Alcalá, Madrid, Spain.[2] Biomedical Networking Research Centre on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN, ISCIII), Madrid, Spain; [3] Ramón y Cajal Health Research Institute (IRYCIS), Madrid, Spain; [4] Departamento de Cirugía, Ciencias Médicas y Sociales, Facultad de Medicina y Ciencias de la Salud, Universidad de Alcalá, Madrid, Spain; [5] Aragon Institute of Engineering Research (I3A), University of Zaragoza, Zaragoza, Spain.

Background: Atraumatic mesh fixation for abdominal hernia repair has been developed to avoid the disadvantages of classical fixation with sutures, which is considered a cause of chronic pain and discomfort. This study was designed to compare, in the short (14 days) and medium term (90 days), the biological and mechanical response of two self-fixing meshes compared to that of a polypropylene (PP) mesh fixed with a cyanoacrylate tissue adhesive in a rabbit model of prosthetic hernia repair.

Methods: Partial abdominal wall defects (6 x 4 cm) were created in New Zealand white rabbits (n = 36) and repaired using a self-adhesive hydrogel mesh (Adhesix™), a self-gripping mesh (ProGrip™) or a PP mesh fixed with cyanoacrylate adhesive (Surgipro™ CA). After 14 and 90 days, the host tissue incorporation, macrophage response and biomechanical strength were examined in each group (n = 6). The statistical comparison of the data (mean ± standard deviation) was performed using Student's paired t test (in the case of a normal distribution) or Wilcoxon's test (in the case of a nonnormal distribution). The test of normality was performed by the Shapiro–Wilk test. Statistical analyses were performed using GraphPad Prism 5 software.

Results: At 14 and 90 days, the ProGrip and Surgipro CA meshes showed good host tissue incorporation; however, the Adhesix implants presented poor integration, seroma formation and a higher degree of shrinkage. The Adhesix hydrogel was completely reabsorbed at 14 days, whereas ProGrip microhooks were observed at all study times. Macrophage response was higher in the ProGrip and Surgipro CA groups at 14 and 90 days, respectively, and decreased over time. At 90 days, the ProGrip implants showed the highest tensile strength values and the Adhesix implants showed the highest failure stretch.

Discussion and conclusions: Meshes with mechanical microgrip self-fixation (ProGrip™) show better biological and mechanical behavior than those with adhesive hydrogel (Adhesix™), in a preclinical model of abdominal hernia repair in rabbits.

Supported by Grants SAF2017-89481-P and PDC2021-121809-I00 from the Spanish Ministry of Science and Innovation, and CIBER-BBN.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Macular edema as a histological factor mediating color perception

García-Palomeque J.C.[1], García-Iñiguez M.[2], García-Gómez N.[1], Molero-Chamizo A.[3] and Salido M.[1]

[1] Histology Department, School of Medicine, Cádiz University, Spain; [2] Ophthalmology Service, Andalusian Health Service, Jerez, Spain; [3] Psychobiology Area, University of Huelva, Spain

Introduction: Macular edema is a clinical sign that is associated with various ophthalmological pathologies and that usually causes decreased vision or scotoma [1]. The morphology and location of the scotoma are helpful in locating ocular injury and its magnitude. However, the evaluation of perceived color is an undervalued clinical sign. The objective of this study is to explore the relationship between histological changes and the chromatic characteristics of the scotoma. The macular lesion can compromise the processing of color by the blue, green, and red cones, as well as the on/off activity of the ganglion cells, through a potential mechanism of ultrastructural modification of retinal histology. Thus, a macular edema can promote the formation of scotomas associated with a primary color.
Methods: In this study we describe a series of clinical cases of patients with macular edema. In one of them, a macular edema was observed in the inferior temporal region, associated with scotoma with perception of blue color. In the other patients, an alteration in vision was detected, without associated scotoma. The patient with scotoma was treated with intraocular glucocorticoid injection after the appearance of the blue scotoma.
Results: A month after starting intraocular therapy, the macular edema remitted, and the bluish scotoma disappeared. Two months after pharmacological intervention, the edema reappeared, as well as the bluish scotoma. Histological changes were observed via coherence computed axial tomography (CAT) and eye fundus examination performed at this stage of the patient's evolution. Changes in the size of the retina were calculated, as well as the angle of refraction of light, using Snell's Law.
Discussion & conclusions: It can be concluded that the visual perception of the blue tone in this patient may be due to an alteration in the trichromatic processing of color attributed to the functionality of the cones affected by the location of the edema [2,3].

[1] Shin YJ, et al. Invest Ophthalmol Vis Sci. 2014;55(1):25-32
[2] Hofer H, et al. J Neurosci. 2005;25(42):9669-79
[3] Cideciyan AV, et al. Hum Gene Ther. 2013;24(12):993-1006

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Involvement of synovial activity in extraosseous endochondral ossification: Histopathological characteristics

García-Palomeque J.C.[1], Hens-Perez A.[2], Molero-Chamizo A.[3] and Larran J.[1]

[1] Histology Department, School of Medicine, Cádiz University, Spain; [2] Pathology Service, Andalusian Health Service, Puerto Real, Cádiz, Spain; [3]Psychobiology Area, University of Huelva, Spain

Introduction: Endochondral ossification is linked to several pathological processes, and it has been shown to be the origin of primary lesions with benign characteristics in synovial locations, such as arthritic joints, tendon sheaths, and bursae. An inflammatory process seems to be related to endochondral ossification in the integrating histologic lesion characteristic of the aortic valve disease [1,2]. Chondral lesions outside the bone tissue are poorly understood. In this study we review several cases of benign chondral tumors with extraosseous lesions related to synovial mechanisms. The objective is to describe these tumors histologically in order to explore their etiopathogenesis and their possible relationship with degenerative aortic stenosis with osteochondral component.
Methods: Seven cases of benign extraosseous tumors were analyzed. The inclusion criteria were the following: benign cartilaginous tumor, location in synovial tissues, and association with inflammatory processes. The histological study was performed by hematoxylin-eosin staining and the pathological diagnosis of the chondral lesion and its histological typing were performed by a specialist pathologist.
Results: Samples from 7 patients showed cartilaginous histological lesions. The diagnostic characteristics of these patients are shown in Table 1. Histological findings revealed cartilaginous tissue with an endochondral ossification profile in the samples.

Table 1.

Tissue	Patients	Diagnosis
Synovial	4	Osteochondromatosis
Tendon	1	Cartilaginous fibroadipose tissue
Skin	2	Chondromyxoid fibrous tissue

Discussion and conclusions: In a series of 7 patients, we observed cartilage lesion in locations with synovial physiology. In all of them, inflammatory processes were evident, which could have triggered cell differentiation mechanisms towards cartilage tissue. These findings have clinical implications since these mechanisms could be pharmacologically modulated. In this vein, in degenerative aortic stenosis, two phases occur around the connective tissue. An initial phase of inflammation and calcification related to the accumulation of saturated fatty acids, and a second phase of endochondral ossification. Our results suggest a parallelism between this pathology and the histological pathogenesis of the chondral lesion [3].

[1] Aikawa E, et al. Human semilunar cardiac valve remodeling by activated cells from fetus to adult: implications for postnatal adaptation, pathology, and tissue engineering. Circulation; 113:1344–1352. 2006
[2] Raja Sivamani Raja Sivamani et al. Chondroid syringoma: case report and review of the literature. Dermatol Online J. 12(5):8. 2006
[3] Artiach G., et al. Inflammation and its resolution in coronary artery disease: a tightrope walk between omega-6 and omega-3 polyunsaturated fatty acids. Kardiol Pol. 78:93–95. 2020

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

FESEM study of talcum powder used in pharma-cosmetics

Delgado R.[1], Crespo P.V.[2], Fernández-González M.V.[1*], García J.M.[1,2], Sánchez-Marañón M.[1], Soriano M.[3], Molinero-García A.[1], Carretero M.I.[4], Márquez R.[5] and Martín-García J.M.[1]

[1] Department of Edaphology and Agricultural Chemistry. University of Granada. Granada (Spain). [2] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [3] Department of Agronomy. University of Almería. Almería (Spain). [4] Department of Crystallography, Mineralogy and Agricultural Chemistry. University of Sevilla. Sevilla (Spain). [5] Scientific Instrumentation Center, University of Granada. Granada (Spain). * corresponding author: mvirginiafernandez@ugr.es

Introduction: Talc refers to mineral raw materials with a high content of the mineral talc [$Si_4O_{10}(OH)_2Mg_3$]. This presents the following properties [1]: moderate specific surface; perfect {001} exfoliation; hardness (H) 1; greasy touch; toughness, flexible, non-elastic; light or white color and insoluble in water. That is why talc can be used for pharmaceutical/cosmetic use as a dermatological and antipruritic protector, deodorant, lubricant, excipient, suspension distributor, and active pharmaceutical ingredient (skin protector in powders, creams and emulsions) [2] [3]; It is also used in pleurodesis as a sclerosant. The skin is the largest organ in the human body, with relevant functions: protective barrier, immunological information, homeostasis, regulates body temperature and water loss, sensory information from the extracorporeal environment, and performs endocrine secretion functions and synthesis, as well as excretion functions through its exocrine glands [4]. In this way, medicinal and cosmetic products, such as talc, dedicated to the healing and care of the skin are of enormous importance for the health sciences.

Material and methods: 65 talcum powders for sale in pharmacies and cosmetic lines in large stores in France, Italy, Norway, Portugal and Spain and purchased on Amazon. Morphological and microelemental analysis of the particles was carried out with a field emission scanning electron microscope (FESEM) and microanalysis (EDX), and image analysis (AI) to evaluate the fibrous character. The samples were prepared on carbon adhesive tape and metallized with carbon.

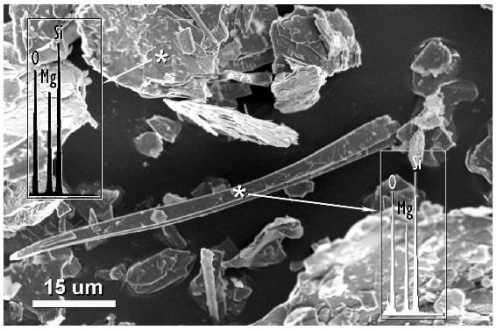


Fig. 1. Talcum powder particles corresponding to the Norway market analyzed with EDX. 1. Laminar talcum powder particle showing exfoliation. 2. Fibrous particle (L/A>5/1µm).

Results and discussion: The mineral talc is observed in all cases as laminar particles with sizes of 50 µm and larger that exfoliate into smaller particles, 5-10 µm. These characteristics show their aptitude for skin care, since these particles will adhere to it easily, exercising, for example, lubricants and deodorants, initially without risk of toxicity. Fibrous particles also appear (L/A>5/1), but its EDX microanalysis, with Mg, Si, O, confirms talc mineralogy, therefore its chemical safety and that on contact with the skin, by body movements, they will crumble easily. The particles of accompanying minerals (carbonates and quartz) are shown with morphologies less favorable for application on the skin (equidimensional and psuedopolyhedral).

Conclusions: The usefulness of FESEM-EDX for the study of pharmaceutical/cosmetic materials is highlighted. This study will be complemented by specific methods of mineralogical and chemical composition (DRX, XRF, ICPmass, for example).

Supported by project EVALTALC (B-CTS-20-UGR20) and Master's Degree in Research, Development, Control and Innovation of Medicines (UGR).

[1] Klein C, Dutrow B. 2017. The 23rd Edition of the Manual of Mineral Science. Wiley, India.
[2] Carretero MI, Pozo M. Appl Clay Sci 46.2009;73–80.
[3] Carretero, MI, Pozo, M. Appl Clay Sci 47.2010; 171-181.
[4] Pawlina W, Ross MH. Histology a Text and Atlas. 8th Ed.Philadelphia: Wolters Kluwer; 2020.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Microbiological analysis of talcum powder for cosmetic use

Del Moral A.[1], Hu Y.[1], Cabezas M.D.[2], Crespo P.V.[3], García J.M.[3], Piqueras A.[4], Fernández-González M.V.[5*] and Delgado R.[5]

1, Department of Microbiology; 2, Department of Pharmaceutical Technology; 3, Department of Histology; 4, Department of Public International Law and International Relations; 5, Department of Edaphology and Agricultural Chemistry. University of Granada, Granada, Spain. * corresponding author: mvirginiafernandez@ugr.es

Introduction: Skin protects the human organism from the external environment, functioning as a selective barrier against microorganisms and chemicals. Scientific knowledge of the skin is the basis of cosmetic art [1]. Talcum powder is a cosmetic product that has been used since ancient times. It is composed of hydrated magnesium silicate (mineral species talc) with varying amounts of associated species, predominantly chlorite, magnesite, calcite and dolomite. Various additives such as perfumes are added to this mineral base. The safety conditions of talcs are established in the quality monographs that make up the pharmacopoeias, which are the official codes that establish the mandatory standards for substances used in products for human use. The European Pharmacopoeia determines its quality specifications as a substance and, depending on its use as a product, it must comply with specific legislation for its marketing [2]. The present work is focused on an evaluation of the microbiological quality of fourteen talc samples purchased directly from pharmacies in Mediterranean-Latin countries (Spain, Portugal, France and Italy) together with talc purchased through the Amazon platform.

Methods: Following the methodology described in the European Pharmacopoeia, the number of aerobic microorganisms and total fungi was determined and the presence of the main cosmetic pathogens referred to in the current legislation was investigated: *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans*. The analysis was completed by scanning electron microscopy studies (FESEM Leo 1530, Zeisscon Gemini EDX Oxford) according to the methodology previously described [3].

Results: Of the fourteen samples tested, thirteen were considered microbiologically fit for consumption while one was found to have contaminants. Specifically, we detected the presence of *E. coli* confirmed by the API10s rapid identification test. On the other hand, the FESEM-EDX study of the talc constituent particles revealed its majority composition in the mineral talc, whose lamellar habit, exfoliation capacity and small particle size make it suitable for cosmetic use. Special cell pre-fixation techniques with glutaraldehyde was also applied have microscopically corroborated the presence of the microorganisms in the contaminated talc.

Discussion & conclusions: The methods followed have proven to be adequate in achieving the stated objectives. No relationship was found between the microbial contamination and the mineral composition of the talc or its preferential use, so we conclude that these are circumstantial facts linked to malpractice, either in the processing and packaging, the storage of the raw materials or the finished product.

Supported by Project EVALTALC (B-CTS-20-UGR20).

[1] Madurga Sanz, M. El papel de la cosmética: excipientes y conservantes. Rev Pediatr Aten Primaria. 2009;11 Supl 15: 81-100.

[2] European Union, 2009. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. In Council, E. P. a. o. t. (Ed.), Regulation (EC) No 1223/2009. Brussels: European Parliament.

[3] Gámiz, E.; Soriano, M.; Delgado Calvo-Flores, G; Párraga Martínez, J; Delgado Calvo-Flores, R. Estudio morfológico de talcos con microscopio electrónico de barrido (sem): aplicaciones farmacéuticas. Ars Pharm. 2002;43(1-2)173-185.

SESSION

SENESCENCE AND AGING

SENESCENCIA Y ENVEJECIMIENTO

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Cellular and molecular changes in tissues derived from the aging process

Syroyid I.[1], Mercado-Sáenz S.[1], Rodríguez Pérez L.M.[1], Sánchez Varo R.[1], Escamilla Sánchez A.[1], Ortega Jiménez M.V.[1,2], Arranz Salas I.[1,2], Peláez González A.[1], López Villodres J.A.[1] and Bermúdez Flores D.[1]

[1] Medical Biology and Histology PAI Reference: CTS429. Department of Human Physiology, Human Histology, Pathological Anatomy and Physical Sport Education. School of Medicine. Malaga University. Spain; [2] Unit of Anatomical Pathology, Virgen de la Victoria University Hospital, 29010 Malaga, Spain;[4] Unit of Radiation Oncology, Virgen de la Victoria University Hospital, 29010 Malaga, Spain

Introduction: This paper is a review of the published literature on cellular aging where the main theories and the latest advances in the mechanisms of senescence are presented.
Objetive: Relevant mechanisms in the aging process at the protein and genetic level are presented.
Method: This review is based, as a thematic guide about aging, in the doctoral thesis of Dr. Silvia Mercado Sáenz, as well as in the book "Mechanisms of aging" by Nozomu Mori and Inhee Mook-Jung.
Based on these fundamental references, a systematic review of articles is carried out in the multidisciplinary database Scopus with the keywords "cell aging".
10 journals with the highest SJR (Scimago Journal Rank) ranking of 2016 were selected, covering the areas of human histology, cell biology, genetics and aging. The filters of "review" articles from the 10 main journals of histology, genetics, cell biology and aging are applied. Articles have been considered between the years 2011-2016.
Results: As a result, in the theoretical field, the preponderance of protein damage as a driver of physiological aging is proposed and in the mechanistic field the hypothesis is proposed that relates the accumulation of damage in proteins of long half-life (NPC and histones) with the induction of senescence in cells aged by the cGAS mechanism.
Discussion and conclusions:

1. There are currently no methods to prolong human life expectancy.
2. Approaches to the study of aging are based on observations of markers of aging in aged individuals. However, the most interesting results seem to come from synthetic biology and genetic engineering.
3. There are cellular problems that are difficult to solve, such as long-lived proteins that pose an obstacle to combating chronological cellular aging.
4. Relating the accumulation of protein damage and the cGAS protein for the detection of cytoplasmic DNA, an aging model is proposed. This model would lead to the senescence of chronologically aged cells or that present accumulation of damage in the proteins of the nuclear pore complex and in the histones that could explain the accumulation of senescent cells in aged organisms.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Role of hepcidin and ferroportin iron transport proteins in Alzheimer's disease

García-Lobo C.[1,2,3], Junceda S.[2,4], Martínez-Pinilla E.[1,2,3], Menendez-Pérez C.[1,2], Álvarez-García E.[1,2], Tolivia J.[1,2,3] and Navarro A.[1,2,3]

[1] GECYEN, Department of Morphology and Cell Biology, University of Oviedo, Asturias, Spain; [2] Instituto de Neurociencia del Principado de Asturias, INEUROPA, Spain; [3] Instituto de Investigación sanitaria del Principado de Asturias, ISPA; [4] Anatomo-pathological Service, Valle del Nalón Hospital, Asturias, Spain

Introduction: Dysregulation of iron balance could be involved in the development and progression of Alzheimer's disease (AD). High concentrations of iron in the brain have been observed both in pathological situations and associated with aging. The proteins related to iron metabolism, at cellular level, that control its entry, exit and storage, would be key in the study of AD. Among these proteins, hepcidin (HCP) and ferroportin (FPN) stand out, due to their direct relationship with iron exportation.

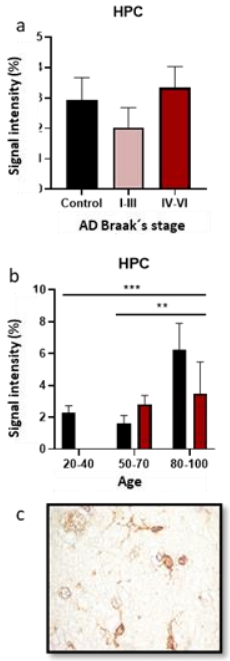


Figure 1. Quantitative and qualitative immunolabeling for HPC.

Methods: The objective of this work was to study the role of these proteins in relation with iron metabolism and the progression of the pathology by qualitative and quantitative immunohistochemical studies.

Results: The results demonstrated that there was no relationship between the tau and beta amyloid proteins, the main histopathological marks of the AD, and the mentioned transporters. Moreover, the expression of FPN did not show a significant relationship with the disease, but with age; same findings were observed for HPC transporter in both situations (Fig. 1a-b y Fig. 2a-b). Interestingly, immunohistochemical labeling for FPN appeared almost exclusively in neurons (Fig. 2c), whereas HPC signal was found in neurons and also in reactive glial cells (Fig. 1c).

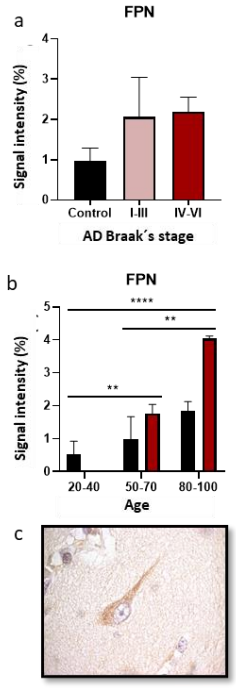


Figure 1. Quantitative and qualitative immunolabeling for FPN.

Discussion & conclusions: Taking into account that brain iron content increases during aging, the data presented here would seem contradictory at first sight. So far, it has been argued that degeneration and death of neurons with age was due, in part, to the excess of intracellular iron. However, we observed an increase of neuronal FPN, a transporter responsible for removing iron from neurons, during lifetime, which would fit with an extracellular iron accumulation leading to an adverse and cytotoxic environment.

With regard to HPC, and based on our results, the increased expression of this transporter could play a double function in nervous system. On one hand, its increase may be related to a role in the degradation of excess FPN. On the other hand, an induction of HPC during neuroinflammation that occurs in AD and aging has also been suggested. The HPC immunosignal that we observed in the microglia would support this last hypothesis.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Role of Mast Cells in Alzheimer's disease

Vázquez-Román V.[1], Montes de Oca-Pineda L.[1], Martín-Lacave I.[1], Fernández-Santos J.M.[1], Ramírez-Ponce M.P.[2], Flores-Cordero J.A.[3] and Alés E.[2]

[1] Department of Normal and Pathological Cytology and Histology, School of Medicine, University of Seville, Spain; [2] Department of Medical Physiology and Biophysics, School of Medicine, University of Seville, Spain; [3] Department of Medical Biochemistry and Molecular Biology and Immunology, School of Medicine, University of Seville, Spain

Background: Alzheimer's disease is a neurodegenerative disorder in which neuroinflammation and microglia activation is involved [1,2]. In this sense, mast cells are cells of the immune system that can traverse the blood-brain barrier under healthy and pathological conditions. They contain abundant secretory granules such as histamine and depending on the number of cells activated, mast cells can play a neuroprotective or neurodegenerative role [3]. Moreover, in the central nervous system (CNS), antihistamines are capable of modulating mast cell degranulation [4]. Currently, there is any information about the number of mast cells depending on the functional state of the brain being the precise location of these cells within the CNS unknown. Therefore, in the present work we carry out a morphometric study of brain mast cells with the aim of knowing if there are differences in their number, size and location between control and Alzheimer brains evaluating the effect of antihistamines.

Methods: A morphometric comparative analysis was carried out on brain samples from different groups of 8-month-old mice, 3 mice per each: 1) a group with induced Alzheimer's disease (E), 2) another with induced Alzheimer's disease treated with ketotifen (ET), 3) a wild-type group as a negative control (C), and 4) another one treated with ketotifen as a positive control (CT). When samples were paraffin-embedded and serially-sectioned a Toluidine Blue staining (0.5% Toluidine blue, Sigma-Aldrich-Germany, in destilled H_2O + 1% glacial acetic acid) was done. The morphometrical analysis was performed and Analysis of variance test (*ANOVA)* was carried out. All measurements were expressed as mean ± standard error of the mean (SEM), being $p \leq 0.05$ considered as significant.

Results: There are morphometrical differences among brain mast cells depending on the group. In Alzheimer group, we found the largest number of mast cells and the smallest cellular size among groups. However, in absence of disease, the number of mast cells remained low. When Ketotifen was used, mast cells increased in size and decreased in number. Mast cells were located either inside or near blood vessels, nevertheless, in the Alzheimer group mast cells were also located immersed the brain parenchyma.

Conclusions: Our results shown that, there are morphometric variations and changes in the degranulation of the brain mast cells depending on the pathophysiological state of the mouse. The neuroinflammatory state induces a degranulation process that triggers neurodegeneration and that can be reversed by antihistamines treatment such as Ketotifen.

Supported by CTS- 439/2017 (Diffuse Neuroendocrine System).

[1] Kempuraj D., et al. (2019) Mast cells in stress, pain, Blood-Brain Barrier, neuroinflammation and Alzheimer's disease. Front Cell Neurosci. Feb 19;13: 54

[2] Forloni G. (2020) Alzheimer's disease: from basic science to precision medicine approach. BMJ Neurol Open. Nov 12; 2(2)

[3] Wernersson S. and Pejler G. (2014) Mast cell secretory granules: Armed for battle. Nat Rev Immunol 2014;14: 478-494

[4] Ramírez-Ponce MP., et al. (2021) Mast Cell Changes the Phenotype of Microglia via Histamine and ATP. Cell Physiol Biochem. Jan 15;55(1):17-32

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Protein homeostasis as a therapeutical target for Alzheimer's disease: analysis in the hippocampus of transgenic mouse models

Sanchez-Varo R.[1,2*], Criado-Alamo E.[1*], Fernandez-Valenzuela J.J.[2], Mercado-Sáenz S.[1], Lopez-Villodres J.A.[1], Escamilla-Sánchez A.[1], Rodriguez-Perez L.M.[1], Arranz-Salas I.[1], Ortega-Jiménez M.V.[1], Alba-Tercedor C.[1] and Gutierrez A.[2]

[1] Department of Human Physiology, Human Histology, Anatomical Pathology and Physical Education. University of Malaga, Malaga, Spain; [2] Department of Cell Biology, Genetics and Physiology/IBIMA/CIBERNED. Faculty of Sciences, University of Malaga, Malaga, Spain.

Introduction: Alzheimer's disease (AD) constitutes the most prevalent form of dementia, being considered one of the global epidemics in the current century, with more than 50 million people affected worldwide. There is neither a cure nor a disease-modifying therapeutic intervention able to slow down the pace of this devastating neurodegenerative condition. Owing to population ageing, it has been estimated that there will be more than 150 million AD patients by 2050. Therefore, there is an urgent need of searching for novel therapeutic targets and treatments.
The main histopathological hallmarks of AD brains are the presence of extracellular amyloid plaques formed by the beta-amyloid (Abeta) peptide, and intraneuronal neurofibrillary tangles composed of hyperphosphorylated tau (phospho-tau) protein. In fact, AD is considered a neurodegenerative proteinopathy. The coexistence of these protein aggregates leads to synaptic damage, neuronal loss and cognitive decline in patients. The dysfunction of the intracellular proteolytic systems (autophagy-lysosomal and ubiquitin-proteasome) has been postulated as a pathological mechanism contributing to the cerebral accumulation of these toxic proteins. The aims of the present work are to verify the involvement of these pathways in two different transgenic mice of this disease, and thus, to better understand the differential impact of Abeta and phospho-tau accumulation on the pathological progression in the hippocampus.
Methods: Cerebral sections containing hippocampus from APP- (APPSL/PS1M146L) and tau- (ThyTau22) based mouse models (from 2 to 18 months of age) were analyzed. Age-matched wildtype (WT) animals were used as controls. Immunohistochemical stainings were performed to evaluate the progression of amyloid deposition and phospho-tau accumulation (AT8 antibody) along aging. Autophagic/lysosomal markers and ubiquitin were also assessed by immunohistochemistry at different pathological stages.
Results: In the amyloidogenic model, we have detected a pathological accumulation of autophagic vesicles, lysosomes and ubiquitin in periplaque dystrophic neurites (aberrant axons and presynapses). Conversely, in the ThyTau22 model, only abnormal accumulation of ubiquitin was detected, mainly located in the somas of principal neurons. In any case, the alteration of protein homeostasis pathways was associated to aging and to the progression of both proteinopathies in these models.
Discussion & conclusions: These results demonstrate significant alterations of the proteolytic pathways in both models of proteinopathy. However, amyloidosis and tauopathy transgenic mice were differentially affected. Preclinical studies in transgenic models able to reproduce the pathogenic mechanisms of patients will allow the identification of novel therapeutic targets and to test effective treatments to stop or modify the course of these diseases. Compounds able to reduce the cerebral burden of toxic proteins might be an alternative pharmacological approach to AD and other tauopathies. Finally, clarifying the basic effects of Abeta and phospho-tau over homeostatic mechanisms would indeed enable the development of alternative therapeutic strategies and drugs targeting pathways related to these proteinopathies.

Supported by CTS-429 (Biology and Histology), and Instituto de Salud Carlos III (ISCiii) of Spain, co-financed by FEDER funds from European Union through grant PI21/00915 (to AG).

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Correlation of the gut microbiota with histological parameters in Alzheimer's

Cortés-Soler A.[1], Mercado Sáenz S.[1], Rodríguez Pérez L.M.[1], Sánchez Varo R.[1], Escamilla Sánchez A.[1], Arranz Salas I.[1,2], Ortega Jiménez M.V.[1,2], Ríos Barranquero M.C.[1], López Villodres J.A.[1] and Bermúdez Flores D.[1]

[1] Medical Biology and Histology PAI Reference: CTS429. Department of Human Physiology, Human Histology, Pathological Anatomy and Physical Sport Education. School of Medicine. Malaga University. Spain; [2] Unit of Anatomical Pathology, Virgen de la Victoria University Hospital, 29010 Malaga, Spain;[4] Unit of Radiation Oncology, Virgen de la Victoria University Hospital, 29010 Malaga, Spain

Objectives: To carry out a current literature review about human gut microbiota, the interplay between the microbiota and the brain via gut-brain axis and the link between gut microbiota and Alzheimer's disease.
Methods: An extensive search has been conducted in articles that describe the link between gut microbiota and the Alzheimer's disease. The search was carried out in the Scopus database with different search criteria: descriptors, key words, topic areas, scientific publications, the article's publication date, etc.
Results: The gut microbiota is a complex assemblage of microorganisms present in the human intestine, interacting with the host through various forms of communication, one of them being the gut-brain axis.
It has been proven that there are gut microbiota alterations in patients with Alzheimer's disease, such as the lowering of the microbiological variety or the predominance of other types of bacteria that are not predominant in healthy people.
The microbiota-brain communication combined with the described alteration suggest lines of treatment focused on modifying the microbiota for patients with Alzheimer's disease in order to improve symptoms.
Conclusions: There is a bidirectional relationship between the gut and the brain. There is a gut microbiological alteration in patients with Alzheimer's disease, yet it cannot be recognized as the main cause of the disease. Nevertheless, this serves as a basis to leave the door open for future research into new treatments.

SESSION

TISSUE ENGINEERING AND ADVANCED THERAPIES

INGENIERÍA TISULAR Y TERAPIAS AVANZADAS

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Effect of perfusion and electrical stimulation on the generation of cardiac tissue *in vitro*

Francisco-Solano E.[1], García-Lorenzana M.[2], Peña-Mercado E.[3], Lara-Rodríguez A.R.[3], Viveros-Moreno N.G.[4], Sanchez-Gomez C.[5] and Beltrán-Vargas N.E.[3]

[1] Posgrado en Ciencias Naturales e Ingeniería, Universidad Autónoma Metropolitana, Cuajimalpa, CDMX, México; [2] Departamento de Biología de la Reproducción, Universidad Autónoma Metropolitana, Iztapalapa, CDMX, México; [3] Departamento de Procesos y Tecnología, Universidad Autónoma Metropolitana, Cuajimalpa, CDMX, México; [4] Doctorado en Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana, Cuajimalpa, CDMX, México; [5] Laboratorio de Biología del Desarrollo y Teratogénesis Experimental, Hospital Infantil de México "Federico Gómez", CDMX, México.

Introduction: Ischemic heart diseases are the leading cause of death in the world, of which acute myocardial infarction (AMI) is the most common [1]. AMI causes the death of heart tissue as a result of reduced blood flow in the coronary arteries. Among the different strategies that seek to regenerate lost tissue in a heart that has suffered an AMI, cardiac tissue engineering (CTE) has promising advances in the creation of cardiac tissue *in vitro* to implant in the damaged area. The CTE uses a cell source, a cell support (scaffold) and a bioreactor to generate culture conditions that favor the maturation of cardiac tissue through the application of perfusion and electrical stimuli. Perfusion increases mass transfer while increasing cell viability [2] and electrical stimulation promotes cell communication, generating a phenotype similar to that of native cardiac tissue with increased expression of structural and functional cardiac proteins (connexin 43, troponins, and tropomyosin) [3]. In this work, the effect of perfusion and electrical stimulation on the maturation of cardiac tissue generated *in vitro* was evaluated.
Methods: The perfusion flow of the bioreactor was calculated based on the oxygen needs of the cells seeded on the scaffolds (construct), the internal mass transfer in the construct, and the shear stress generated by the flow. Neonatal rat cardiomyocytes were used and seeded on alginate-chitosan scaffolds functionalized with alginate-coated gold nanoparticles (AuNp+Alg). Cells were cultured for 3 days under static culture conditions plus 4 days of dynamic culture (perfusion + electrical stimulation). The constructs were processed with routine histological technique, they were stained with Hematoxylin-Eosin. A qualitative and quantitative analysis of the generated spheroids was performed and the expression of tropomyosin (TPM) was quantified by immunohistochemistry. Differences between groups were evaluated with a one-way ANOVA followed by Tukey's test, with a value of $p<0.05$.
Results: The calculated flow of 0.5 mL/min associated with a shear stress of 1.8 dyne/cm^2 helps to generate cardiac spheroids significantly larger than static culture, and a flow of 1.5 mL/min (1979 ± 325.1 μm^2 vs. 1309 ± 99.3 μm^2 vs. 1023 ± 99.3 μm^2). Furthermore, TPM expression is significantly higher at 0.5 mL/min than in static culture. A voltage of 5 V helps to generate spheroids three times larger than a voltage of 3 V (1594 ± 275.9 μm^2 vs. 572 ± 73 μm^2).
Discussion & conclusions: Perfusion and electrical stimulation promote cell organization and integration, generating more cardiac spheroids. A flow of 0.5 mL/min and electrical stimuli of 5 V help to generate larger spheroids, with higher expression of TPM. The results suggest that the bioreactor designed by our group allows the generation of functional cardiac tissue, which can be used in AMI.

The authors appreciate the support and facilities provided by CONACyT, HIMFG and SECTEI.

[1] Rana, J. S., Khan, S. S., Lloyd-Jones, D. M., and Sidney, S. (2021). Changes in mortality in top 10 causes of death from 2011 to 2018. *Journal of general internal medicine*, *36*(8): 2517-2518.
[2] Cheng, M., Moretti, M., Engelmayr Jr, G. C., and Freed, L. E. (2009). Insulin-like growth factor-I and slow, bi-directional perfusion enhance the formation of tissue-engineered cardiac grafts. *Tissue Engineering Part A*, *15*(3): 645-653.
[3] MOON, Sung-Hwan, et al. Electrically stimulable indium tin oxide plate for long-term in vitro cardiomyocyte culture. *Biomaterials research*, 2020, vol. 24, no 1, p. 1-8.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Polyethylene glycol hydrogels including full-length vitronectin mimic neuroblastoma microenvironment

Monferrer E.[1,2], Dobre O.[3], Trujillo S.[4], Azevedo González Oliva M.[3], Trubert-Paneli A.[3], Acevedo-León D.[5], Noguera R.[1,2] and Salmerón-Sánchez M.[3]

[1] Department of Pathology, Medical School, University of Valencia-INCLIVA Biomedical Health Research Institute, 46010 Valencia, Spain; [2] Low Prevalence Tumors, Centro de Investigación Biomédica En Red de Cáncer (CIBERONC), Instituto de Salud Carlos III, 28029 Madrid, Spain; [3] Centre for the Cellular Microenvironment, Advanced Research Centre, University of Glasgow, Glasgow, United Kingdom, G116WE; [4] INM – Leibniz Institute for New MaterialsCampus D2 2, 66123 Saarbrücken, Germany; [5] Clinical Analysis Service, Hospital Universitario Dr. Peset, 46017 Valencia, Spain

Introduction: The components of the extracellular matrix (ECM) and their mechanical properties regulate cancer development, aggressiveness, and metastasis, so their incorporation into 3D cell culture systems is required to develop appropriate cancer models. Polyethylene glycol (PEG) hydrogels are versatile platforms that allow stiffness regulation and incorporation of full-length ECM proteins. Since high territorial vitronectin (VN) expression in human tissues samples leads to a poor prognostics outcome in neuroblastoma (NB) patients, we designed adaptable VN/PEG hydrogels to recapitulate part of the native NB microenvironment. In this work, we assessed VN/PEG hydrogel fitness as a NB model by evaluating PEGylated VN retention and bioactivity, SK-N-BE(2) cell viability and subsequently stiffness measurement by nanoindentation. Finally, we imaged the relation between PEGylated and cellular VN to assess the behavior of SK-N-BE(2) in the system.
Methods: Full-length VN was first PEGylated with MAL-PEG-SVA at 1:4 mass ratio to covalently bond the protein into the PEG network. Focal adhesion formation in SK-N-BE(2) neuroblastic cells were analyzed to check whether PEGylation reduced VN bioactivity. Briefly, cells were seeded onto VN and PEGylated VN substrates and cultured in free FBS media for 24h. Then, cells were fixed, stained, imaged by fluorescence microscopy and later evaluated with CellProfiler. VN/PEG hydrogels were formed using different amounts of 4-arm-PEG-MAL (3 wt% or 10 wt%) and a final concentration of 500 µg mL^{-1} of PEGylated VN. A mixture of VPM protease degradable peptide and PEG-dithiol at 1:9 ratio was used to crosslink the hydrogels. PEGylated VN retention into the system was evaluated after 72 h by anti-VN immunofluorescence staining in cryosections and later imaging and quantification by QuPath. Nanoindentation measurements to assess hydrogel stiffness were performed with Chiaro Nanoindenter. SK-N-BE(2) cells were encapsulated into the hydrogels during fabrication at $2x10^6$ cells mL^{-1} and cultured for 7 days. Cellular viability was tested by Live/Dead assay on day 1, 5 and 7 and images were analyzed by FIJI to further evaluate cluster growth and density. VN produced and secreted by cells was identified by confocal imaging and ELISA colorimetric assay performed on collected media during cell culture period.
Results: PEGylated VN was properly retained within the PEG network, being as bioactive as the native VN. VN/PEG hydrogels recapitulated a small range of physiological stiffness, from 0.42 ± 0.09 kPa to 4.12 ± 2.14 kPa. Interestingly, VN incorporation did not modify hydrogel stiffness, but increased cell degradation capability. 80% of the cells remained viable after 7 days and aggregated in clusters. Despite no differences in cluster size were depicted, cluster density was lower in stiff hydrogels. SK-N-BE(2) continued synthesizing VN even in VN rich conditions.
Discussion & conclusions: The experiments carried out herein demonstrate the suitability of the engineered VN/PEG models to mimic NB behavior and further suggest them as potential drug testing platforms.

Supported by Asociación Fundación Española contra el Cáncer, JAP-AECC (2018/150); ISCIII (FIS) and FEDER (European Regional Development Fund), grant number PI20/01107; CIBERONC (contractCB16/12/00484); EPSRC Programme Grant (EP/P001114/1).

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Decellularized adipose tissue extracellular matrix for three-dimensional stem cell culture and bone tissue engineering

Ibarretxe G.[1], Luzuriaga J.[1], García-Urkia N.[2], García-Gallastegui P.[1], Irastorza I.[1], Etxeberria I. [1], Salvador J. [1], Olalde B.[2], Unda F.[1], Pineda J.R.[1,3] and Madarieta I.[2]

[1] Department of Cell Biology and Histology, University of the Basque Country (UPV/EHU), Leioa, Spain; [2] TECNALIA Basque Research & Technology Alliance, Donostia-San Sebastián, Spain; [3] Achucarro Basque Centre for Neuroscience Fundazioa, Leioa, Spain

Introduction: Current disease models used for drug screening and development do not accurately emulate all facets of human tissues. 2D cell cultures are some of the most employed pre-clinical in vitro models, but they often involve cell seeding on polystyrene surfaces, such as culture flasks (T-flasks) or Petri dishes, which are unable to reproduce the real complexity and 3D structure found in the human body [1]. Thus, conventional 2D cell culture systems over polystyrene present obvious limitations to what concerns the testing of biologically active compounds, and the generation of tailored micro-tissue grafts for tissue engineering therapies. With this in mind, there is an active ongoing search for new biomaterials that support the long-term culture of human stem cells, and their controlled differentiation to diverse adult tissue lineages in 3D matrices. Bone tissue engineering is one such discipline that demands the development of new reliable 3D culture approaches for the generation of mineralized bone tissues of tunable sizes and shapes, which recapitulate adult bone tissue dynamics *in vivo*.
Methods: We made 3D cultures of human Dental Pulp Stem Cells (hDPSCs), a multipotent stem cell type with osteogenic capacity, seeded in porcine and human Decellularized Adipose Tissue (pDAT and hDAT) matrices, which had been processed by freeze-drying method from solutions from 0.25% to 1.5% (w/v), to obtain porous solid foams with different stiffness, as described in [2]. We assessed the differentiation of hDPSCs to osteoblasts by ALP staining, as well as by the expression of bone-matrix proteins, by RT-qPCR and immunofluorescence (IF). Bone matrix deposition on 3D hDPSC cultures was assessed by Alizarin Red staining, and the ultrastructure of the generated bone micro-tissue was assessed by transmission electron microscopy (TEM).
Results: hDPSCs presented good viability and proliferation rates in both hDAT and pDAT solid foams. The seeded hDPSCs could be induced to differentiate to osteoblasts, as shown by their increased levels of ALP activity and the expression of bone matrix proteins Osteocalcin/BGLAP and Osteonectin/SPARC. Furthermore, hDPSCs could terminally differentiate to mature osteoblasts which eventually mineralized the whole solid foam by intramembranous ossification, as assessed by TEM and Alizarin Red staining. We found there were statistically significant differences in osteogenesis between 3D sister cultures in hDAT vs pDAT [3], and when cells were grown in solid foams with different stiffness, being 0.25% pDAT the most effective combination.
Discussion & conclusions: These results suggest that hDAT and pDAT are very versatile biomaterials for bone tissue engineering, because osteogenesis by hDPSCs could be modulated by factors such as the species origin of the DAT, and its concentration. Because of their bioinductive properties, biocompatibility and tailored formulation possibilities, these biomaterials could be an option for the bioengineering market, and the design of personalized medicine strategies in the fields of traumatology, orthopaedics and craniomaxillofacial surgery.

Supported by the Basque Government (ELKARTEK program; PLAKA KK- 2019-00093), MICINN/AEI/10.13039/50110001103 (PID2019-104766RB-C21 & MINECO RYC-2013-13450), and UPV/EHU (PPGA20/22).

[1] Breslin & O'Driscoll 2013. Drug Discov. Today; 18 (5-6), 240-249.
[2] Cicuéndez et al. 2021. Int. J Mol. Sci. 22(8): 3847.
[3] Luzuriaga et al. Eur. Cell. Mater. 2022; 43, 112-129.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Potential usefulness of decellularized sturgeon cartilage in tissue engineering

Ortiz-Arrabal O.[1,2], Carmona R.[3], González-Gallardo C.[4], García-García O.D.[1,2], Chato-Astrain J.[1,2], Sánchez-Porras D.[1,2], Carriel V.[1,2], Garzón I.[1,2], Campos A.[1,2] and Alaminos M.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Department of Cellular Biology, Faculty of Sciences, University of Granada, Spain; [4] Servicio de Oftalmología, Hospital Universitario San Cecilio, Granada, Spain

Introduction: Development of novel biomaterials with appropriate biocompatibility is one of the goals of current tissue engineering. In this context, natural scaffolds have several advantages as compared to other biomaterials and decellularization of natural products demonstrated to be a promising method to obtain biocompatible and highly biomimetic biomaterials [3,4]. In this work, we decellularized sturgeon cartilage and investigated its potential usefulness in cornea and cartilage tissue engineering.

Methods: Sturgeon cartilage was obtained and decellularized following protocols based on a combination on different detergents and an enzymatic treatment. The decellularization efficiency was evaluated by quantifying DNA and DAPI and hematoxylin-eosin staining. Decellularized cartilage was then recellularized with cell cultures of 4 different types of mesenchymal stem cells (adipose tissue, Wharton Jelly, bone marrow and dental pulp) and kept *in vitro* for 4 weeks and *in vivo* for 60 days. Then, samples were analyzed using histological, histochemical and immunohistochemical methods.

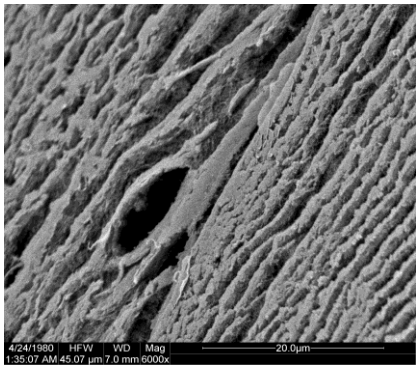


Fig. 1. SEM analysis of decellularized sturgeon cartilage.

Results: The general structure of sturgeon cartilage was properly preserved after the decellularization protocol (Fig. 1). Once recellularized, *ex vivo* results showed that sturgeon cartilage promotes cell adhesion and proliferation of several types of MSC and no changes were found in extracellular matrix composition. *In vivo* assays revealed that sturgeon cartilage was safe for the host animals, and remained stable at least 60 days and encapsulated by host connective tissue with no cellular infiltration. Moreover, all cell types were able to increase the content of collagen, especially when adipose MSC were used ($p<0.05$). These substitutes also triggered a pro-regenerative reaction mediated by M2 macrophages.

Discussion & conclusions: The decellularization protocol used in this work allowed us to maintain the structure of the sturgeon cartilage unlike other previous protocols that such as lyophilization or solubilization. Decellularized biomaterials showed good *ex vivo* and *in vivo* biocompatibility and tended to remain stable after MSC recellularization with several types of cells. These results support the use of decellularized sturgeon cartilage as a novel biomaterial in tissue engineering of tissues requiring strict physical and optical properties such as the human cornea or the cartilage.

Supported by the Spanish Plan Nacional de Investigación Cientifica, Desarrollo e Innovación Tecnológica (I+D+i) of the Spanish Ministry of Science and Innovation through grant FIS PI20/0317 and ICI21-00010 (NANOULCOR) from Instituto de Salud Carlos III, co-financed by the European Regional Development Fund (ERDF). Supported by grant PI-0257-2017 from Consejería de Salud y Familias, Junta de Andalucía, Spain.

[1] Tuan R.S., Chen A.F. and Klatt B.A. (2013). Cartilage Regeneration. J. Am. Acad. Orthop. Surg. 21(5): 303-311

[2] Kabir W., Di Bella C., Jo I., Gould D. and Choong P.F.M. (2021). Human Stem Cell Based Tissue Engineering for *In vivo* Cartilage Repair: A Systematic Review. Tissue Eng. Part B Rev. 27(1): 74-93

[3] Gómez-Florit M., et al. (2020). Natural-Based Hydrogels for Tissue Engineering Applications. Molecules. 25(24): 5858

[4] Taylor D.A., Sampaio L.C., Ferdous Z., Gobin A.S. and Taite L.J. (2018) Decellularized matrices in regenerative medicine. Acta Biomater. 74: 74-89

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Synthesis and characterization of gold nanoparticles to functionalize alginate-chitosan scaffolds for cardiac cell culture

Marcial-Becerril M.R.[1], Campos-Terán J.[2], Arrollo-Maya I.J.[2], Ruiz. J.C.[2], Rodríguez-Reyes F.[3] and Beltrán-Vargas N.E.[2]

[1] Posgrado en Ciencias Naturales e Ingeniería, División de Ciencias Naturales e Ingeniería, Universidad Autónoma Metropolitana Unidad Cuajimalpa, CDMX, México. [2] Departamento de Procesos y Tecnología, División de Ciencias Naturales e Ingeniería, Universidad Autónoma Metropolitana Unidad Cuajimalpa, CDMX, México; [3] Licenciatura en Ingeniería Biológica, División de Ciencias Naturales e Ingeniería, Universidad Autónoma Metropolitana Unidad Cuajimalpa, CDMX, México

Introduction: Acute myocardial infarction (AMI) is one of the main causes of death in the world. During AMI, the loss of cardiomyocytes due to cell death and their inability to regenerate prevents the repair of damaged tissue, causing heart failure [1]. Cardiac tissue engineering (CTE) by seeking the generation of artificial tissue through in vitro cell culture on scaffolds developed with biodegradable and biocompatible materials, can help in the repair of damaged tissue. However, there have been limitations in the scaffolds used as they fail to imitate certain natural properties of native tissues, so their functionalization, particularly with nanomaterials, has being studied [2,3]. In CTE, the use of gold nanoparticles (AuNp) due to their conductive properties, their easy functionalization and adjustment to different sizes and shapes, makes them ideal for the culture of cardiac cells [4,5]. In this work, the synthesis and characterization of AuNp is carried out and its interaction with alginate-chitosan scaffolds (Alg-CS) used in cardiomyocyte culture is evaluated.
Methods: AuNp synthesis was performed following the procedure established by Topete et al. [6], with some modifications. PLGA cores were generated and a part of them was coated with alginate. Subsequently, the nuclei with and without alginate were attached to gold nanoseeds and their growth was measured. The generated AuNp and AuNp+Alg were evaluated by dynamic light diffraction (DLS) to determine their size and Z-potential, and were observed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to determine their morphology. Then the NPs were added to Alg-CS scaffolds manufactured by our group and swelling tests were carried out for 7 days to determine their effect, evaluating the Np previously diluted in ultrapure water (milli-Q) and phosphate buffered saline (PBS).
Results: AuNp and AuNp+Alg with sizes of 81.72 ± 2.57nm and 81.18 ± 1.31nm were obtained; and Z potential values of -30.47 ± 3.65 mV and -29.27 ± 1.79 mV, respectively. The micrographs obtained by SEM confirm the dispersion and size of the NPs; while the micrographs obtained by TEM indicate that the generated NPs have a star shape. Maximum swelling values of 3830.8% and 3811.3% were obtained for the Alg-CS scaffolds functionalized with AuNp and AuNp+Alg diluted in Milli-Q and 2535.8% and 2349.3% for those diluted in PBS, respectively.
Discussion & conclusions: The results indicate that the generated Np are within the range used in CTE, with a morphology that can favor the growth of cardiomyocytes. The Np diluted in milli-Q present higher percentages of swelling of the scaffolds, which favors the absorption of nutrients for cell growth.

[1] Qasim, M., Arunkumar, P., Powell, H. M. and Khan, M. (2019). Current research trends and challenges in tissue engineering for mending broken hearts. Life Sci. 229, 233–250.
[2] Tamimi, M., Rajabi, S. and Pezeshki-Modaress, M. (2020). Cardiac ECM/chitosan/alginate ternary scaffolds for cardiac tissue engineering application. Int. J. Biol. Macromol. 164, 389–402.
[3] Tallawi, M., Rosellini, E., Barbani, N., Cascone, M. G., Rai, R., Saint-Pierre, G. and Boccaccini, A. R. (2015). Strategies for the chemical and biological functionalization of scaffolds for cardiac tissue engineering: a review. J. R. Soc. Interface 12(108):20150254.
[4] da Silva, A. B., Rufato, K. B., de Oliveira, A. C., Souza, P. R., da Silva, E. P., Muniz, E. C., Vilsinski, B. H. and Martins, A. F. (2020). Composite materials based on chitosan/gold nanoparticles: From synthesis to biomedical applications. Int. J. Biol. Macromol. 161, 977–998.
[5] Yadid, M., Feiner, R. and Dvir, T. (2019). Gold Nanoparticle-Integrated Scaffolds for Tissue Engineering and Regenerative Medicine. Nano Lett. 19(4), 2198–2206.
[6] Topete, A., Alatorre-Meda, M., Villar-Álvarez, E. M., Cambón, A., Barbosa, S., Taboada, P. and Mosquera, V. (2014). Simple control of surface topography of gold nanoshells by a surfactant-less seeded-growth method ACS Appl. Mater. Interfaces 6(14), 11142–11157.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Study of the swelling and degradation capacity of alginate-chitosan scaffolds for their application in tissue engineering

Rodríguez-Reyes F.[1], Ruiz. J.C.[2], Marcial-Becerril M.R.[3], Campos-Terán J.[2] and Beltrán-Vargas N.E.[2]

[1] Licenciatura en Ingeniería Biológica, División de Ciencias Naturales e Ingeniería, Universidad Autónoma Metropolitana Unidad Cuajimalpa, CDMX, México; [2] Departamento de Procesos y Tecnología, División de Ciencias Naturales e Ingeniería, Universidad Autónoma Metropolitana Unidad Cuajimalpa, CDMX, México; [3] Posgrado en Ciencias Naturales e Ingeniería, División de Ciencias Naturales e Ingeniería, Universidad Autónoma Metropolitana Unidad Cuajimalpa, CDMX, México.

Introduction: Tissue engineering is an emerging field in the medical area that links medicine with engineering, biology, physics and chemistry, to develop tissue constructs in order to restore or correct a defect in tissues or organs based on the use of cells, and biologically active molecules, physical and chemical signals that mimic the biological conditions of tissue and biomaterials, which support cells and provide appropriate conditions for their growth and proliferation [1]. In this work, the manufacture of alginate-chitosan scaffolds is proposed and the physicochemical characterization was carried out to evaluate their ability to absorb nutrients (percentage of swelling) and their percentage of degradation.

Methods: Alginate-chitosan scaffolds of 0.75-1.25% w/v were made by lyophilization, with 30 min of crosslinking with calcium gluconate. The swelling limit of the hydrogels in ultrapure water (Milli-Q) and in phosphate buffered saline (PBS) was calculated gravimetrically in triplicate. The structure and porosity of the scaffolds and their percentage of degradation at 3 months were observed by scanning electron microscopy.

Results: Maximum swelling values of 2000% and 2500% at 60 min were obtained, and of 2345% and 2931% in Milli-Q and PBS at 7 days, respectively. The swelling percentages change to 2000% and 3500% at 60 days, respectively, and reach values of 3000% and 3500% at 90 days. The percentages of degradation at 7 days were 20% and 27% in Milli-Q and PBS, respectively; increase to 23% and 30% at 30 days, and increase to 75% and 80% at 60 days, which remain stable up to 90 days.

Discussion & conclusions: The results indicate that the elaborated scaffolds allow a good absorption of liquids and that this capacity increases with time, which is very favorable for its application in tissue engineering. In addition, degradation remains low during the first month, which would favor cell adhesion and proliferation, and at 3 months 80% of the hydrogels are lost. The results suggest that the proposed scaffolds have adequate physicochemical properties for their application in tissue engineering.

The authors appreciate the support provided by SECTEI.

[1] Chandra, P. K., Soker, S., & Atala, A. (2020). Tissue engineering: current status and future perspectives. Principles of Tissue Engineering, 1–35. doi:10.1016/b978-0-12-818422-6.00004-6

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

In vivo biocompatibility test of a novel chitosan-alginate cardiac patch

Viveros-Moreno N.G.[1], García-Lorenzana M.[2], Peña-Mercado E. [3], Sánchez-Gómez C.[4], Salazar-García, M.[4], Chávez-Oviedo V.L.[5], Francisco-Solano E.[6] and Beltrán-Vargas N.E.[3]

[1]Doctorado en Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana, Cuajimalpa, México; [2] Departamento de Biología de la Reproducción, División de Ciencias Biológicas y de la Salud. Universidad Autónoma Metropolitana, Iztapalapa, México; [3]Departamento de Procesos y Tecnología, División de Ciencias Naturales e Ingeniería, Universidad Autónoma Metropolitana, Cuajimalpa, México; [4] Laboratorio de Biología del Desarrollo y Teratogénesis Experimental, Hospital Infantil de México "Federico Gómez", CDMX, México, [5] Licenciatura en Ingeniería Biológica, Universidad Autónoma Metropolitana, Cuajimalpa, CDMX, México; [6] Posgrado en Ciencias Naturales e Ingeniería, Universidad Autónoma Metropolitana, Cuajimalpa, CDMX, México

Introduction: Acute myocardial infarction (AMI) is one of the primary cardiovascular diseases which lead to cardiomyocyte ischemia, ventricular hypertrophy, heart failure and death [1]. Therefore, the search for therapies for remodeling infarcted tissue has been prioritized. One therapy for cardiac regeneration is the use of engineered cardiac tissue by seeding cardiac cells into scaffolds. The scaffolds should bring structural support without triggering an immune response. A wide variety of biomaterials have been explored as scaffolds including synthetic and natural polymers [2]. However, natural biomaterials have been reported to improve the mechanical and biological properties of the patches. In this context, the Department of Processes and Technology of the UAM Cuajimalpa developed an alginate-chitosan (Alg-CS) cardiac patch that shows favorable swelling, degradation, and compatibility. This could increase the viability of the patch when implanted in the infarcted myocardium. However, its safety must be evaluated. The aim of this work was to analyze the biocompatibility of an Alg-CS patch by subdermal implantation on the back of the Wistar rat.
Methods: A small incision was made in the interscapular area of female and male Wistar rats under aseptic and anesthetic conditions. A small pocket was formed between the skin and the muscle, where an Alg-CS sponge (6 mm in diameter) was placed. The incision was closed with surgical glue. At 1, 3, 7 and 25 days after subdermal implantation, tissue was obtained for histological analysis. For basic histomorphological evaluation and for identification of collagen fibers, paraffin sections were stained with hematoxylin and eosin, or by Mason's Trichrome technique. To detect vessels formation and inflammation process TNFα and PECAM-1 were evaluated. Microphotographs were obtained with Aperio ImageScope software.
Results: Histological analysis reveals that host tissue's reaction is consistent with foreign body response. Integration and biodegradation gradually progress over time. The therapeutic patch presented favorable biocompatible characteristics, such as the formation of a capsule of highly vascularized connective tissue, whose thickness decreased after 25 days of implantation; the formation of blood vessels within the scaffold; and progressive degradation of the scaffold without immunological exacerbation. No differences between sex were observed.
Discussion & conclusions: During foreign body reaction, 4 main stages were identified: protein adsorption, acute inflammation, chronic inflammation, and collagen deposition around the implanted biomaterial, which were clearly identifiable in the histological analysis. The resolution of this reaction depends on morphology, degradation, porosity, and shape of the scaffold. The combination of Alg-CS can form a scaffold that has the ability to swell in the presence of body fluids, in addition to modulate the inflammatory process, stimulate the proliferation of fibroblasts and the production of collagen fibers, promote the development of blood vessels, and improve the quality of scar tissue. These characteristics allow adequate communication between the host tissue and the implant. With these results, it will be possible to implant the patch in a murine model of AMI. This will allow to evaluate the functionality of the patch and scale it to the clinical setting.

The authors appreciate the support and facilities provided by CONACyT, HIMFG and SECTEI.

[1] Khan, MA, et al. Global Epidemiology of Ischemic Heart Disease: Results from the Global Burden of Disease Study. Cureus, 2020;12(7): p. e9349
[2] Portillo Esquivel LE, et al. ACS Biomater. Sci. Eng. 2021;7(3):1000-1021

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

A functional, structural and histochemical comparison between human hyaline and elastic cartilage microtissues for tissue engineering applications

Sánchez-Porras D.[1,2], Losilla-Martínez J.M.[3], Durand-Herrera D.[1,2], Ávila-Fernández P.[1], Sola M.[1,2], Ortiz-Arrabal O.[1,2], Crespo P.V.[1,2], Rodríguez I.A.[1,4], Campos F.[1,2] and Carriel V.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Servicio de Cirugía Plástica y Reparadora, Hospital Universitario Virgen de las Nieves, Granada, Spain; [4] Cátedra "B" de Histología y Embriología, Facultad de Odontología, Universidad Nacional de Córdoba, República Argentina; Contact: davidsp@ugr.es; vcarriel@ugr.es

Introduction: Current cell therapy and tissue engineering strategies have not shown efficient clinical results in the treatment of cartilage injuries. Low cell proliferation and chondrocyte dedifferentiation observed during *in vitro* expansion are typically found as the most relevant limitations [1]. In this regard, new cell sources and culture techniques are needed to promote the biomimicry needed to overcome these limitations [2]. For this reason, the aim of this *ex vivo* comparative study was to evaluate the efficacy of two sources of human chondrocytes (hyaline and elastic) during the generation of chondrogenic microtissues (MT) for use in tissue engineering.

Methods: MT were generated using agarose microchips using hyaline and elastic chondrocytes cultured with chondrogenic culture media, and results were evaluated for 28 days at the structural, biochemical and histological levels.

Results: The hyaline and elastic MT were found to be composed of abundant viable and functional cells. WST-1 average values were considerably higher in hyaline MT (Fig. 1). Histology confirmed the formation of stable microtissues with randomly distributed cells. In addition, histochemistry confirmed the maintenance of the chondrogenic profile and an increase in proteoglycan synthesis over time, especially in the hyaline microtissues. In contrast, collagen staining was positive in both cell types, but slightly higher in elastic microtissues.

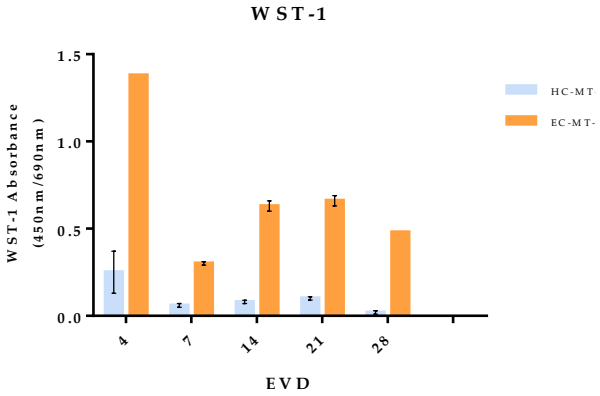


Fig. 1. *Average and standard deviation of metabolic activity of hyaline and elastic cartilage microtissue.*

Discussion: This *ex vivo* study demonstrated the suitability of both types of human chondrocytes to generate viable, functional and stable microtissues for cartilage tissue engineering applications. However, our structural, functional and histological analyses demonstrated differential behavior between hyaline and elastic chondrocytes, with both being suitable for use in biofabrication processes [3]. Future *in vivo* studies are still needed to determine the potential usefulness of these chondrogenic MT in the repair of damaged elastic and hyaline cartilage.

Fundings: This study was supported by grant PI-0257-2017 from Consejería de Salud y Familias, Junta de Andalucía, Spain, and CTS-115 (Tissue Engineering Group).

[1] Garcia-Martinez L, et al. (2017) Histochem Cell Biol. 2017;147(1):83-95
[2] Durand-Herrera D et al. (2018) Histochem Cell Biol. 2018;150(4):379-93
[3] Sánchez-Porras D. et al. (2021) Biomedicines. Mar 12;9(3):292

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Decellularized sclerocorneal limbus scaffolds for tissue engineering applications

Sánchez-Porras D.[1,2], González-Gallardo C.[2,3], Ortiz-Arrabal O.[1,2], Ávila-Fernández P.[1], Blanco-Elices C.[1,2], Sola M.[1,2], Fernández-Valadés R.[1,2,3], Crespo P.V.[1,2], Campos F.[1,2] and Alaminos M.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Division of Ophthalmology, University Hospital Virgen de las Nieves, Granada, Spain; [4] Division of Pediatric Surgery, University Hospital Virgen de las Nieves, Granada, Spain

Introduction: The sclerocorneal limbus is an anatomical structure of the eye that comprises the limbal stem cell niche that is responsible for the corneal epithelium homeostasis [1]. Advanced therapies may provide an effective treatment to replace the dysfunctional sclerocorneal limbus, but the complex structure of the stem cell niche makes it difficult to recreate a suitable biological substitute. Xenograft tissue decellularization may be a promising technique to generate new biomimetic limbal substitutes. The aim of this study is to evaluate the efficiency of different decellularization methods applied to the sclerocorneal limbus.

Methods: Porcine sclerocorneal limbi were obtained from a local slaughterhouse. Limbi were carefully dissected to remove all remnants of iris, conjunctiva and other peripheral tissues. Then, half of the limbi were diametrically dissected to preserve only the anterior half of the limbus (anterior hemilimbus or HL), whereas the rest of the limbi were used complete (full limbus or FL). FL and HL were subjected to four different decellularization protocols. In all protocols, a common first stage of osmotic shock with distilled water was applied. Protocol 1 (P1) was based on the use of SDS detergent. P2 applied a hypertonic NaCl medium. P3 applied a combination of SDS, Triton X-100 and SDC detergents and nucleases, and P4 combined a trypsin treatment with the detergents and nucleases of protocol 3. Decellularization efficiency was analyzed by DNA quantification and hematoxylin-eosin histological analysis. Tissue structure and composition were evaluated by histological and histochemical analysis.

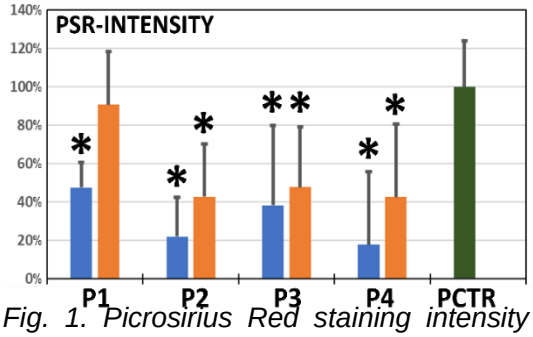


Fig. 1. Picrosirius Red staining intensity quantification (collagen content) for native control and new sclerocorneal substitutes using different decellularization protocols.

Results: DNA quantification analysis demonstrated the effectiveness of all decellularization protocols to remove nuclear contents in FL and HL. All protocols were able to generate substitutes with adequate optical properties, although P1 showed the best transmittance results when applied to HL. Interestingly, HL decellularized with P1 retained a collagen pattern expression that was more similar to controls in terms of fibers alignment and staining signal intensity than other decellularization protocols.

Discussion & conclusions: These results suggest that the use of limbal xenografts is a good alternative to generate decellularized scaffolds with potential usefulness in tissue engineering. Decellularization protocols based on the use of SDS succeeded in generating sclerocorneal limbi with adequate optical properties and extracellular matrix composition. These decellularized scaffolds could be useful for the generation of sclerocorneal limbi in the laboratory by tissue engineering.

Supported by grant CSyF PI-0086-2020 from the Consejería de Salud y Familias, Junta de Andalucía, Spain, and grant B-CTS-504-UGR20 (proyectos de I+D+i en el marco del Programa Operativo FEDER Andalucía 2014-2020, University of Granada and Consejería de Transformación Económica, Industria, Conocimiento y Universidades). Cofinanced by the European Regional Development Fund (ERDF) through the “Una manera de hacer Europa” program. Supported by the Spanish Plan Nacional de Investigación Cientifica, Desarrollo e Innovación Tecnológica (I+D+i) of the Spanish Ministry of Science and Innovation through grant FIS PI20/0317 and ICI21-00010 (NANOULCOR) from Instituto de Salud Carlos III, co-financed by the European Regional Development Fund (ERDF).

[1] Sánchez-Porras D, et al. Pharmaceutics. 2021;13:1718
[2] Chato-Astrain J, et al. Tissue Eng. Regen. Med. 2020;14:789–806

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Influence of melanocytes in corneal re-epithelialization

Pérez-Garrastachu M.[1], Romo-Valera C.[1], Agirrebengoa-Arrieta A.[1], Arluzea J.[1], Boyano M.D.[1,2] and Andollo N.[1,2]

[1]Department of Cell Biology and Histology, School of Medicine and Nursing, University of the Basque Country, Sarriena, S/N, 48940 Leioa, Spain. [2]Biocruces Bizkaia Health Research Institute, Cruces Plaza S/N, 48903 Barakaldo, Spain

The cornea is a highly differentiated avascular transparent tissue in charge of focusing light into the retina. Its great degree of specialization implies that the corneal epithelium must be replenished from the corneal periphery, where the esclero-corneal limbus is placed. This well-organized tissue region characterized by the palisades of Vogt, contains the limbal crypts, where the limbal epithelial stem cells (LESCs) reside. LESCs constantly supply daughter cells to the corneal epithelium in order to maintain a healthy cornea.

LESCs are in need of support cells that create a specific stem-cell niche. In addition, this is the only corneal region that is vascularized and highly innervated. The limbal melanocytes are considered essential players in the homeostasis of the limbal niche, as they not only protect LESCs from UV radiation but also regulate their growth and the maintenance of the stemness properties [1, 2].

Limbal stem cell deficiency (LSCD) is a chronic ocular surface pathology originated by a loss of the LESC population, due to a deregulation or a dysfunction of the limbal niche. Clinically shows conjunctivalization of the cornea, chronic inflammation, and recurrent episodes of epithelial defects that can lead to corneal ulceration and scarring, resulting in serious visual morbidity.

The purpose of this work was to study the role of melanocytes in the corneal re-epithelialization. Preliminary results on primary cultures of limbal cells showed that, depending on the culture conditions, limbal melanocites tended to be oriented towards limbal epithelial progenitor cells. Then, scratch wound healing assays were performed on co-cultures of melanocytes and human corneal epithelial cells, varying the proportion of melanocytes and epithelial cells, the culture surface coating and the culture medium. Results show that re-epithelialization is sensitive to the assayed parameters.

The authors thank tissue banks from Navarra and Barcelona for corneas, and the University of the Basque Country for funding (GIU 20/035).

[1] Polisetti, et al. The Ocular Surface 22 (2021) 172–189
[2] Romo-Valera, et al. EER 211 (2021) 108720

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Histoarchitecture of skeletal muscle reconstructed with autologous adipose tissue in volumetric muscle loss, after performing physical exercise

López-Espejo M.E.[1,2], Gil-Belmonte M.J.[1,2,3], Peña-Toledo M.A.[2,4,5], Agüera A.[1], Leiva-Cepas F.[1,2,3,5], Moreno-Llorente C.D.[1], Ruz-Caracuel I.[1,2,6], Jimena I.[1,2,3] and Peña-Amaro J.[1,3]

[1]Department Morphological Sciences. Section of Histology, Faculty of Medicine and Nursing, University of Córdoba, Spain; [2]Muscle Regeneration Group, University of Cordoba, Cordoba, Spain; [3]Department of Pathology, Torrecardenas Hospital, Almeria, Spain; [4]Dementia and Multiple Sclerosis Unit, Neurology Service, Reina Sofia University Hospital, Cordoba, Spain. [5]Maimonides Institute for Biomedical Research IMIBIC, Reina Sofia University Hospital; University of Cordoba, Cordoba, Spain; [5]Department of Pathology, Reina Sofia University Hospital, Cordoba, Spain. [6]Department of Pathology, Ramon y Cajal University Hospital, IRICYS, Madrid, Spain.

Background: The reconstruction of a muscle injury due to volumetric loss by implanting autologous adipose tissue is a valid strategy that favors the neoformation of skeletal muscle. However, this new muscle has abnormal features. In the present study, we examined whether physical exercise could improve histoarchitectural features.
Methods: Twenty-three adult male Wistar rats were used, divided into six experimental groups: three sedentary (normal control (NC), regenerative control (CR), and with volumetric loss injury and fresh autologous adipose tissue transplantation (FAT)) and three subjected to a treadmill exercise protocol (exercise-rehabilitated CN (RCN), exercise-rehabilitated CR (RCR), and exercise-rehabilitated TAF (RTAF). Grip strength was assessed and, after sacrifice, the tibialis anterior muscles were removed and processed for study in light microscopy with histological, histochemical and immunohistochemical techniques.The histomorphometric analysis included the assessment of: number of fibers, cross-sectional area, minor diameter, number of fibers with central nuclei, number of disoriented fibers and percentage of fibrosis.
Results: Overall, muscle strength tended to increase in the exercise-rehabilitated groups relative to the control groups ($p=0.086$). Exercise rehabilitation also tended to decrease both the percentage of regenerated fibers with central nuclei in the RCR vs CR group ($p=0.053$) and the proportion of fibers with spatial disorientation in the RTAF vs TAF group ($p=0.056$). The percentage of the area with fibrosis was significantly higher in the RCR, TAF and RTAF groups compared to the RCN group ($p<0.05$).
Discussion and conclusion: Rehabilitation with physical exercise for 5 weeks tended to normalize muscle histology in a volume loss injury model reconstructed by TAF implantation, making it faster, more complete, and more functional, but did not counteract the intense fibrosis associated with these injuries.

Acknowledgments: Supported by grant PS-2020-946 from Consejería de Salud y Familias, Junta de Andalucía, Spain.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Limited regenerative response of skeletal muscle in the reconstruction of a volumetric loss using a porous collagen matrix scaffold

Leiva-Cepas F.[1,2,3,4], Gil-Belmonte M.J.[1,2,5], Jimena I.[1,2,3], Agüera A.[1,2], Cantarero I.[1,2], Ruz-Caracuel I.[1,2,6], Villalba R.[3,7] and Peña-Amaro J.[1,2,3]

[1]Muscle Regeneration Group, University of Cordoba, Cordoba, Spain; [2]Department Morphological Sciences. Section of Histology, Faculty of Medicine and Nursing, University of Córdoba, Spain; [3]Maimonides Institute for Biomedical Research IMIBIC, Reina Sofia University Hospital; University of Cordoba, Cordoba, Spain; [4]Department of Pathology, Reina Sofia University Hospital, Cordoba, Spain; [6]Department of Pathology, Ramon y Cajal University Hospital, IRICYS, Madrid, Spain; [6]Department of Pathology, Torrecardenas Hospital, Almeria, Spain; [7]Center for Blood Transfusion, Tissues and Cells, Córdoba, Spain

Background: The volumetric muscle loss injury constitutes the paradigm of an impaired regeneration process. To supply this volumetric defect, scaffolds of all types have been used with some degree of success. The purpose of this study was to assess the use of a porous collagen matrix for the reconstruction of a volumetric muscle loss defect.
Methods: Twenty-eight adult male Wistar rats were divided into four groups: normal control, regenerative control, fibrosis control, porous collagen matrix (PCM) implanted. An experimentally induced defect was created at the tibial anterior muscle and replaced with a same-sized fragment of PCM. Animals were sacrificed at 21, 28 and 60 days. Then, histological, histochemical and immunohistochemical techniques were used. Cytoarchitectural and distribution features were analyzed semi-quantitatively. A quantitative analysis followed by statistical analysis was also performed for the histomorphometry parameters.
Results: At 21 and 28 days, MyoD + and myogenin + nuclei and regenerative muscle fibers were observed in the area near the implant. At 60 days, skeletal muscle neoformation was evident in the implant area, although the muscle fibers showed important cytoarchitectural abnormalities and histological features indicative of innervation failure.
Discussion and conclusion: This study provides evidence that PCM did not inhibit regeneration but promoted a hindered regeneration at remodelling phase. Further studies are needed to improve the performance of the PCM as a viable source of scaffolding for a volumetric muscle loss defect.

Acknowledgments: Supported by CTS-985 Muscle Regeneration Group and by grant PS-2020-946 from Consejeria de Salud y Familias, Junta de Andalucia, Spain.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Amniotic membrane effects onto pathological wound healing: a mechanistic assessment

Liarte S.[1], Bernabé-García A.[1], Rodríguez-Valiente M.[1,2], Pipino C.[3], Stelling-Férez J.[1,4], Moraleda J.M.[5], Pandolfi A.[3], Castellanos G.[2] and Nicolás F.J.[1]

[1] Regeneration, Molecular Oncology and TGFß. IMIB-Arrixaca, Murcia, Spain; [2] Chronic Wounds and Diabetic Foot Ulcer unit, Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain; [3] Department of Medical, Oral and Biotechnological Sciences, University G. d'Annunzio Chieti-Pescara, Italy; [4] Department of Nutrition and Food Technology, UCAM, Guadalupe, Murcia, Spain; [5] Hematology, Haematopoietic Transplantation and Cellular Therapy, University of Murcia, Murcia, Spain

During wound healing, the migration of keratinocytes onto newly restored extracellular matrix aims to finalize the healing process. Transforming growth factor (TGF)-ß plays a very important role at this step; indeed, its altered expression can lead into chronification of the wound, delaying or even preventing its natural healing. The application of Amniotic Membrane (AM) onto wounds “halted” pathological wounds (e.g., chronification, diabetic foot ulcer) has proven very successful at restarting wound healing, in particular re-epithelialization. Cryopreserved AM from elective cesareans and a human keratinocyte cell model (HaCaT) were used together to investigate AM effect on gene and protein expression involved in these results. By using wound-healing scratch assay the effect of AM on cell migration was assessed, while the expression of key cell migration proteins at the wound edge was determined by using cyto-immunofluorescence staining. In order to recreate the chronic environment of the wound, we have developed models using (i) long term serum starved TGF-ß treated HaCaT cells (SSTC-HaCaT) and (ii) human umbilical vascular endothelial cells from gestational diabetic mothers (GD-HUVEC), as they recapitulate characteristics of both pathological granulation tissue and diabetes affected vessel’s endothelium. *In vitro* results were compared data obtained from patient’s chronic wound tissue sections. When applied on epithelial cells, AM activates several important signaling pathways required for migration and proliferation. Importantly, AM management of TGFß signaling on cell proliferation together with stimulation of migration produced the right balance of parameters necessary for the keratinocytes to successfully re-epithelialize. Moreover, an important role of AM in the cytoskeleton and focal adhesions restructuring has been envisaged. Additionally, the analysis of several parameters, upon application of AM on GD-HUVEC, suggest an improvement of critical features indicative of a vascularization improvement. Finally, AM treatment of SSTC-HaCaT produced a clear improvement of several parameters related with the resetting of a successful wound healing: migration, gene/protein expression, proliferation, etc. The comparison to patient´s chronic wounds offered interesting similarities. So, in short, AM constitutes a powerful agent for the healing of complicated/chronified wounds. The precise knowledge of the molecular mechanisms involved on the phenomena will allow us to improve its application and to look for successful AM based ameliorated strategies for future therapeutic application.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Effects of Mesenchymal Stem Cells-derived secretomes to promote tissue regeneration in vivo

Blanco-Elices C.[1,2,3], Cases-Perera O.[1,4], Ortiz-Arrabal O.[2,3], Chato-Astrain J.[2,3], González-Gallardo C.[3,5], Alaminos M.[2,3], Oyonarte S.[2,3,6], Sánchez-Quevedo M.C.[2,3], Martín-Piedra M.[2,3] and Garzón I.[2,3]

[1] Doctoral program in Biomedicine, University of Granada, Spain; [2] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [3] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [4] Department of Plastic Surgery, University Hospital Virgen de las Nieves, Granada, Spain; [5] Division of Ophthalmology, University Hospital Virgen de las Nieves, Granada, Spain; [6] Network for Transfusional Medicine, Cells and Tissues, Granada, Spain

Introduction: Tissue Engineering can be applied to the treatment of diseases for which a curative treatment is not currently available. An emergent promising strategy is the use of mesenchymal stem cell (MSC)-derived secretomes, which could be enriched in numerous growth factors, cytokines, proteases and other pro-regenerative proteins synthetized by these cells that are involved in cell adhesion, proliferation and tissue regeneration [1]. In the present work, we have evaluated the usefulness of different types of secretomes derived from cultured MSC to enhance tissue regeneration in laboratory animals.

Methods: We carried out an *in vivo* analysis on adult 12-week-old Wistar laboratory rats subjected to surgical damage. Full-thickness tissue defects were generated in each animal under general anesthesia. These injuries were then treated with two different MSC-derived secretomes obtained from cultured Adipose Tissue Stem Cells -ADSCs- and umbilical cord Wharton's Jelly Stem Cells -WJSCs-. Dulbecco's Modified Eagles Medium (DMEM) was used as control. The different compounds (secretomes or DMEM) were applied on the injuries every 2-3 days. Animals were followed for four weeks and images of each injury were taken at sequential follow-up times.

Results: The analysis of wound healing treated with MSC-derived secretome showed some differences among the experimental groups. Injuries treated with WJSCs-derived secretome tended to show enhanced *in vivo* wound healing and tended to re-epithelize faster than control defects. However, animals treated with ADSCs-derived secretome did not differ from controls. No side detectable effects (necrosis, infection, tumorigenesis, etc.) were observed after secretome treatment in any of the study groups.

Discussion & conclusions: Our results confirm that treatment with MSC-derived secretome is safe for the animals and the host tissues. In addition, WJSCs-derived secretomes could be considered as a promising approach able to improve wound healing and regeneration of the human skin, cornea, oral mucosa and palate.

Supported by the Spanish Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I) of the Spanish Ministry of Science and Innovation (Instituto de Salud Carlos III), grants FIS PI18/0331, FIS PI21/0980, FIS PI20/0317, ICI19/00024 (BIOCLEFT) and ICI21/00010 (NANOULCOR), and by grants PE-0395-2019 and PI-0442-2019 from the Consejería de Salud y Familias, Junta de Andalucía, Spain. Addition support was provided through grant B-CTS-450-UGR20 (proyectos de I+D+i en el marco del Programa Operativo FEDER Andalucía 2014-2020, University of Granada and Consejería de Transformación Económica, Industria, Conocimiento y Universidades), and cofinancing was provided from the European Regional Development Fund (ERDF) through the "Una manera de hacer Europa" program.

[1] Baez-Jurado E, et al. Mol. Neurobiol. 2019;56(10):6902–6927

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Clinical translation of a bioartificial model of human palate for the treatment of children with cleft palate

Chato-Astrain J.[1,2], Garzón I.[1,2], Blanco-Elices C.[1,2], España-López A.[3], Ortiz-Arrabal O.[1,2], Martín-Piedra M.A.[1,2], Sánchez-Quevedo M.C.[1,2], Rodríguez I.A.[1,4], Fernández-Valadés R.[1,2,3,5] and Alaminos M.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Craniofacial Malformations and Cleft Lip and Palate Management Unit, University Hospital Virgen de las Nieves, Granada, Spain; [4] Cátedra "B" de Histología y Embriología, Facultad de Odontología, Universidad Nacional de Córdoba, República Argentina; [5] Division of Pediatric Surgery, University Hospital Virgen de las Nieves, Granada, Spain

Introduction: Treatment of children with cleft palate in challenging due to the lack of healthy palate mucosa available for repairing the defect. In addition, currently available surgical techniques use to denudate the remaining palatal bone, which is typically associated to growth and development defects of the maxillofacial bone resulting in significant hypoplasia. In this regard, our research group previously generated a biological substitute of the palate mucosa by tissue engineering called BIOCLEFT, and we demonstrated that this substitute could contribute to repair palatal defects while preventing maxillofacial misdevelopment and hypoplasia in animal models [1,2]. In the present work, we describe the clinical translation of this model of bioartificial palate generated by tissue engineering by fulfilling the regulations established by the Spanish Medicines Agency (AEMPS).

Methods: After the initial description of the BIOCLEFT model, this bioartificial tissue was characterized ex vivo using histological and histochemical methods to determine its potential to reproduce the structure of the human palate mucosa. Then, this structure was grafted in vivo in newborn rabbits subjected to a surgical damage of the palate mucosa, and its biosafety, biocompatibility and its potential to prevent the growth and development alterations caused by bone denudation were analyzed. With all this preclinical information, we designed a clinical trial to evaluate the usefulness of the BIOCLEFT model to prevent the maxillofacial bone alterations typically found in cleft palate children treated with the gold-standard technique.

Results: Our preclinical results showed that the palate mucosa generated by tissue engineering using palate mucosa epithelial and stromal cells was biomimetic with the native palate mucosa. This structure consisted of a stromal substitute with biomaterials and stromal cells, and a stratified epithelium on top able to express crucial markers of palate mucosa differentiation, including several cytokeratins. In vivo analyses confirmed the biocompatibility and biosafety of BIOCLEFT and demonstrated the potential of this tissue to prevent misdevelopment of maxillofacial bones. All these results allowed us to obtain a preclinical assessment report form AEMPS, which led us to design a clinical trial to evaluate its potential in children with cleft palate. Preliminary results confirm the possibility to generate the BIOCLEFT model as an advanced therapy medicinal products for use in the clinical trial.

Discussion & conclusions: Our preclinical results obtained ex vivo and in vivo demonstrate the potential usefulness of the BIOCLEFT model to treat patients with cleft palate. The use of this regenerative medicine approach could contribute to improve the results of current surgical techniques applied to the clinical treatment of cleft palate children. Implementation of this clinical trial will determine the real usefulness of this approach.

This study was supported by the Spanish Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I) of the Spanish Ministry of Science and Innovation (Instituto de Salud Carlos III), grant ICI19/00024 (BIOCLEFT). Co-funded by FEDER funds, European Union.

[1] Martín-Piedra MA, et al. Histochem Cell Biol. 2017;147(3):377-388
[2] Fernández-Valadés-Gámez R, et al. Biomed Mater. 2016;11(1):015015

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Osteoblasts provoke a pro-inflammatory shift in macrophage cells lineage

Toledano-Osorio M.[1], Jacho D.[3], Osorio R.[1,2], Toledano M.[1,2] and Yildirim-Ayan E.[3]

[1] Biomaterials Group, Department of Stomatology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs,GRANADA, Granada, Spain; [3] Bioengineering Department, University of Toledo, Toledo, Ohio, USA.

Introduction: Guided tissue/bone regeneration is essential for periodontal and peri-implant bone augmentation. An osteoconductive and osteoinductive clinical environment will determine the existence of new bone formation and will induce the differentiation of stem cells into osteoblasts. However, immune response is also critical in bone healing; macrophages play pivotal and dynamic roles in bone regeneration. Therefore, the crosstalk between the immune and bone-forming cells is of utmost importance. The objective of the present research was to ascertain the effect of osteoblasts differentiation and activity on macrophages activation and cytokine liberation.

Methods: Osteoblasts from an immortalized human fetal osteoblastic cell line were encapsulated within 3 mg/ml collagen type I solution along with PBS and an osteogenic culture media. 3D cell-encapsulated collagen scaffolds were incubated in a standard cell culture incubator at 37° C and 5% CO_2. After four days, osteoblast activity was probed by alkaline phosphatase production and Alzarin red. After 7 days, osteoblasts culture media was collected and mixed (50%) with Roswell Park Memorial Institute (RPMI) 1640 medium. The human pro-monocytic cell line U937 (ATCC, Manassas, VA, USA) were differentiated into macrophages (M0) and activated toward a pro-inflammatory (M1) phenotype. Two groups were used for the immunomodulation study: 1) M1 and 2) M1 CM (macrophages cultured in the mixed 50% RPIM1640 / 50% osteoblast conditioned media). The expression of anti-inflammatory *CCL-18, IL-10*, and *CD163* and pro-inflammatory *IL1 B, TNFa* and *MMP3* markers were studied. Differences between groups were assessed by Student t test ($p < 0.05$).

Results: Main results are displayed in Figure 1.

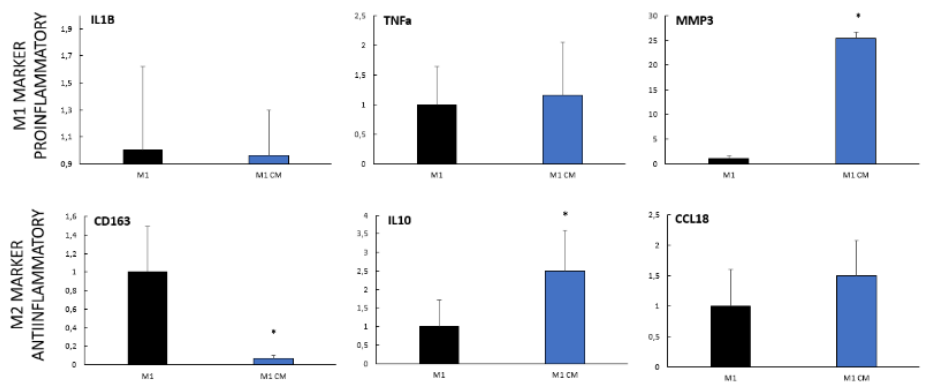


Figure 1: Pro- and anti-inflammatory markers expression on macrophages cultured in the presence or absence of osteoblast conditioned medium (CM).

Discussion & conclusions: Conditioned media collected from active osteoblasts evokes in macrophages a pro-inflammatory response. It may be hypothesized that this reaction would also influence the cross-talk between the immune and bone-forming cells, modulating in turn osteoblasts and osteoclasts activity.

Supported by Ministry of Economy and Competitiveness and European Regional Development Fund, grant number PID2020-114694RB-I00. M.T.-O. holds a FPU fellowship from the Ministry of Universities (FPU20/00450).

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORAL PRESENTATION

Mechanism of action of electric current in the treatment of scars in an animal model

López Morales A.[1], Valera-Garrido F.[2,3] and Santafe M.M.[1]

[1]Unit of Histology and Neurobiology, Department of Basic Medical Sciences, Faculty of Medicine and Health Sciences, Rovira i Virgili University, Reus, Spain. [2]Servicio de Fisioterapia MV Clinic. Pozuelo de Alarcón, Spain. [3]Getafe CF, Madrid, Spain

Background: Percutaneous Needling Electrolysis (PNE) is a minimally invasive technique that consists of the application of a galvanic current through solid needles. Among other applications, PNE is used in the treatment of pathologies related to scars, although its mechanism of action on scars is not yet clear. On the one hand, a simple mechanical action may be involved by the introduction of the needle into the scar. On the other hand, electrolysis of extracellular fluid releases NaOH, which can digest the scar. The objective of this study is to describe the main mechanism by which PNE releases scar tissue from other tissues.

Methodology: A scar was created in Swiss mice by injection of 10% acetic acid subcutaneously in the area of the levator auris longus (LAL) muscle. Ten days later, the tissue was removed. The sample was submerged in oxygenated Ringer's solution that kept them alive. Dry needling (DN) was performed on a group of 5 muscles and PNE on another group of 5 muscles. In both cases, the needle was introduced using a micromanipulator with an inclination of 60º. Needles (AGU-PUNT®, Barcelona, Spain) of 0.32x40mm were used. Using the Physio Invasiva 2.0 device (Prim SA, Mostoles, Spain), a current of 3mA was applied for 3 seconds. Each muscle underwent a maximum of 15 insertions. The holes created by the needle insertion were visualized with a scanning electron microscope (SEM; (FEI ESEM Quanta 600 FEG). Photomicrographs were taken at 450x magnification. The areas of the holes were evaluated using the Digimizer program (MedCalc Software Ltd). Belgium). Data were analyzed with SPSS version 21.0 (SPSS, Inc., Chicago, IL, USA). The results are expressed as mean and standard deviation (SD), considering the 95% Confidence Interval. We used the two-tailed Welch's t-test for unpaired values because our variances were not equal. Differences were considered significant at $P < 0.05$

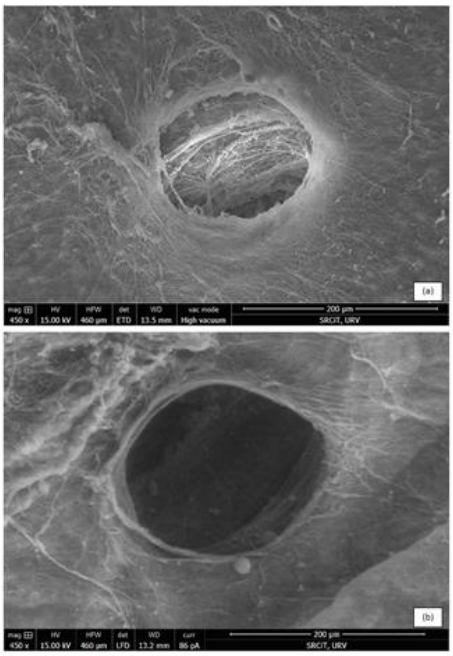


Figure 1. Examples of images obtained with SEM. Images of the perforations made with dry needling (DN, a) and with galvanic currents (PNE, b). Notice that the edges of the lower image are softer than those of the upper one.

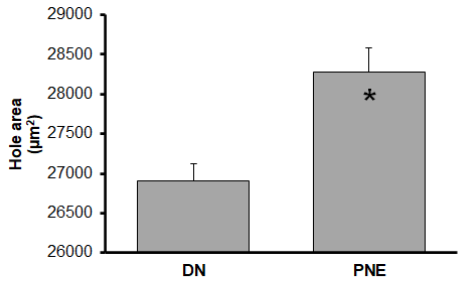


*Figure 2. Hole areas. DN, dry needling. PNE, galvanic current. *p<0.05. n=57 DN holes tested. N=50 PNE holes evaluated.*

Results: Microphotographs showed that the holes created by the simple puncture have irregular edges. However, the holes created by PNE have coagulated edges (Figure 1). Furthermore, the average areas generated by PNE were significantly larger than DN (Figure 2).

Conclusion: The coagulated edges and larger area suggest that the main mechanism of action in scar release with galvanic currents is through the action of NaOH.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Analysis of tissue engineering translation in Spanish institutions. A bibliometric approach on clinical trials and patents

Martin-Piedra M.A.[1,2], Santisteban-Espejo A.[3,4], Campos F.[1,2], Sola M.[1,2], Blanco-Elices C.[1,2], Oyonarte S.[1,2,5], Crespo P.V.[1,2], Garzón I.[1,2], Sánchez-Quevedo M.C.[1,2] and Campos A.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Department of Pahology, Hospital Universitario Puerta del Mar, Cádiz, Spain; [4] Biomedical Research and Innovation Institute of Cadiz, Cádiz, Spain; [5] Network for Transfusional Medicine, Cells and Tissues, Granada, Spain

Introduction: Tissue engineering (TE) is an interdisciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes that restore, maintain, or improve tissue function [1]. Particularly, the main objective of TE is the clinical translation of bioartificial tissues to clinical practice; hence, assessment of the status of TE translation reflects the degree of development of this research area. In this sense, a bibliometric evaluation of documents closely related to translational effort, such as clinical trials or patents could be a feasible approach for the assessment of TE development.
Methods: Information search was performed using a validated query for the retrieval of TE documents ("TISSUE ENGINEER*" OR "TISSUE-ENGINEER*") [2]. ClinicalTrials.gov database, from the National Library of Medicine was used to retrieve the participation in clinical trials focused on TE and registered in Spain. The National Clinical Trial number (NCT number), trial title, condition, intervention, institution ante he odid phase of development were collected for each one of the obtained clinical trials. Moreover, patent information was retrieved from the European Patent Office (EPO) online database. As in clinical trials analysis, a validated TE search query was applied, and only Spanish patents were submitted to analysis of participant institutions.
Results: A total of 6 clinical trials that evaluate TE applications were recorded from Catalonia, Andalusia and Murcia. All the studies were Phase I-II clinical trials mainly focused on the diseases of the bone and joints (4 of 6 clinical trials). Furthermore, a study from the Germans Trias i Pujol Hospital, and a study from the Andalusian Initiative for Advanced Therapies evaluated the use of TE applications for the treatment of myocardial infarction and corneal lesions, respectively. A total of 27 patents were retrieved and solicited by Spanish scholar or health institutions. Universidad of Granada is involved in 6 patents, followed by Banc de Sang i Teixits and Universidad Complutense de Madrid and Consejo Superior de Investigaciones Científicas. Among health institutions, Servicio Andaluz de Salud highlighted with 4 patents.
Discussion & conclusions: One of the basic goals of TE is the translation of research advances to clinical practice through clinical trials and patents. The development of clinical trials demonstrates that TE research aims an effective clinical translation, especially in those diseases in which conventional treatments are ineffective or have failed. Also, Spanish institutions have made a great translational effort through their involvement in patents. It is relevant to mention that this translational effort was made both by scholar institutions (universities, public or private research centers) and clinical institutions (hospitals and health services). It is remarkable that 22% of retrieved patents were requested by Andalusian institutions, such as the University of Granada or the Andalusian Health Service, suggesting a great awareness of this region with translational research of TE.

Supported by CTS-115 (Tissue Engineering Group).

[1] Langer R, Vacanti J. Science. 1993;260(5110):920-26
[2] Santisteban-Espejo A, et al. J Med Internet Res. 2021; 23(11):e25394

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Analysis of the scientific production of tissue engineering in otorhinolaryngology

Martin-Piedra M.A.[1,2], Padilla-Cabello J.[3,4], Moral-Muñoz J.A.[5,6], Blanco-Elices C.[1,2]., García-García O.D.[1,2], Crespo P.V.[1,2], Oyonarte S.[1,2,7], Fernández-Valadés R.[1,2,8], Campos A.[1,2] and Garzón I.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Ph.D. programme on Biomedicine, University of Granada, Granada, Spain; [4] Service of Otorhinolaryngology, Hospital Universitario San Cecilio, Granada, Spain; [5] Biomedical Research and Innovation Institute of Cadiz, Cádiz, Spain; [6] Department of Nursery and Physiotherapy, University of Cádiz, Cadiz, Spain; [7] Network for Transfusional Medicine, Cells and Tissues, Granada, Spain; [8] Division of Pediatric Surgery, University Hospital Virgen de las Nieves, Granada, Spain

Introduction: Tissue engineering is a discipline in a continuous process of consolidation [1]. In this work, we have analyzed the development of this discipline in the specialty of otorhinolaryngology. One of the aspects analyzed was the relative scientific production on this specific area in each country, evaluating the research effort of the different countries in the development of tissue engineering in the otorhinolaryngology field.

Methods: The set of papers in common between tissue engineering and otorhinolaryngology published so far was identified, with a total of 343 papers. The Relative Priority Index (RPI), also known as relative specialization index (RSI), was applied in order to analyze the production in a specific area of research of each country taking into account its global publications. The value of RPI = 100 indicates that a country's research priority in a field is comparable to that of other countries. A value above 100 indicates a higher interest than the other and a value below 100 indicates a lower priority than other countries. For a more contextualized analysis, we also used the Adjusted Index (AI), which is calculated on the basis of each country's GDP per capita (25). This index is obtained by dividing the total number of documents in a country by its Gross Domestic Product (GDP) per capita, multiplied by 100, obtaining a value between 0 and 1.

Results: The highest RPI was observed in Malaysia (439.44), Germany (297.97) and Austria (275.28). Other countries with an RPI greater than 100 are South Korea (261.41), Japan (225.18) and the USA (146.92), a value that indicates their superior relative interest compared to the rest of the countries. The countries with the highest AI were the USA (0.26), Germany (0.13) and Japan (0.11). On the other hand, the positions of Malaysia (0.07) and South Korea (0.06), which ranked fourth and fifth respectively, stood out.

Discussion & conclusions: While the USA has a greater number of published papers, when the RPI is evaluated, it can be found that other countries make a greater relative effort in research and production in this field. In this sense, Malaysia and Austria, unlike Germany, are not among the five most productive countries in scientific publications. However, these countries produce a greater number of papers on tissue engineering in otorhinolaryngology with respect to their total scientific output, which implies a greater interest in this area of study. Other countries with a higher relative interest than the rest are South Korea, Japan and the USA. On the other hand, when the AI was evaluated, the USA, Germany and Japan lead the ranking, which correlates directly with the main producers of articles. This result provides further evidence of the correlation between the wealth (GDP per capita) of a country and its scientific output. Again, Malaysia and South Korea stand out as countries with high tissue engineering research in the field of otorhinolaryngology, despite having a lower GDP per capita than the other countries.

Supported by CTS-115 (Tissue Engineering Group).

[1] Mhashilkar AM, Atala A. Advent and maturation of regenerative medicine. Curr Stem Cell Res Ther. 2012;7(6):430-445

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Impact and collaboration in the scientific production of tissue engineering and otorhinolaryngology: a bibliometric analysis

Martin-Piedra M.A.[1,2], Padilla-Cabello J.[3,4], Santisteban-Espejo A.[5,6], Moral-Muñoz J.A.[6,7], Ávila-Fernández P.[1], España-López A.[8], García J.M.[1,2], Sánchez-Quevedo M.C.[1,2], Rodríguez I.A.[1,9] and Campos A.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Ph.D. programme on Biomedicine, University of Granada, Granada, Spain; [4] Service of Otorhinolaryngology, Hospital Universitario San Cecilio, Granada, Spain; [5] Department of Pathology, Hospital Universitario Puerta del Mar, Cádiz, Spain; [6] Biomedical Research and Innovation Institute of Cadiz, Cádiz, Spain; [7] Department of Nursery and Physiotherapy, University of Cádiz, Cadiz, Spain; [8] Craniofacial Malformations and Cleft Lip and Palate Management Unit, University Hospital Virgen de las Nieves, Granada, Spain; [9] Cátedra "B" de Histología y Embriología, Facultad de Odontología, Universidad Nacional de Córdoba, República Argentina

Introduction: Tissue engineering (TE) is a relatively recent discipline that has undergone a rapid evolution in recent decades. This area of research focuses its efforts on the development of tissues and organs artificially in order to obtain a substitute for the replacement of those damaged or absent tissues, without the comorbidity derived from obtaining tissue from other parts of the patient's body. This is particularly relevant in reconstructive surgery of the head and neck. In this sense, otorhinolaryngology (ORL), as the medical-surgical speciality of reference in head and neck surgery, can assume the different lines of application of TE techniques. It is necessary to evaluate the current state of this emerging area of research in the ORL speciality using bibliometric methods to analyse, from a descriptive and social point of view, the impact of the production of TE in ORL.
Methods: Firstly, the corpus of documents that are common to the areas of knowledge of ORL and TE published between 1900 and 2020 was identified, using the Web of Science (WOS) database. This corpus was first analysed from a descriptive point of view, using the information obtained from WoS for each article, classifying them according to document type, country of publication, language of publication, publication journal, authorship of the articles, institution related to authorship and, finally, citations. Secondly, an analysis of the social structure of this set of publications was carried out using VOSviewer software, which allows us to draw up maps of collaboration between countries and institutions.
Results: A total of 343 documents were identified and analysed, corresponding to publications with a clinical profile in the field of ORL including TE applications. The number of publications per year shows a fluctuating evolution, while the number of citations shows a pattern of growth over the years, demonstrating a growing interest in this type of publications. The distribution of authorship and journals of publication revealed a clear imbalance, with a minority being responsible for the majority of papers, according to Bradford's Law. There is a clear predominance of some countries in the production in this area, such as the United States or Germany. The institutions that play the most relevant role in the scientific production of TE and ORL were identified, such as the University of California or the University of Kyoto, and the collaboration networks that are established between them.
Discussion & conclusions: Tissue engineering is still at an early stage in otorhinolaryngology. As a research area, it is in a phase of active growth, where there are still no signs of consolidation and synthesis. Further institutional efforts are needed to promote progress in this field and to establish greater networks.

Supported by CTS-115 (Tissue Engineering Group).

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Effectiveness of maslinic acid as an inductor of keratinocyte proliferation in tissue engineering

Ortiz-Arrabal O.[1,2], Chato-Astrain J.[1,2], Crespo P.V.[1,2], Martín-Piedra M.A.[1,2], Rodríguez I.A.[1,3], Sánchez-Quevedo M.C.[1,2], Garzón I.[1,2], Mesa-García M.D.[2,4], Gómez-Llorente C.[2,4] and Alaminos M.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Cátedra de Histología B, Universidad de Córdoba, República Argentina; [4] Deparment of Biochemistry and Molecular Biology II, School of Farmacy, Granada, Spain and Biomedical Research Center, Institute of Nutrition and Food Technology "José Mataix", University of Granada, Granada, Spain

Introduction: Treatment of severely burnt patients with bioengineered skin substitutes [1] is limited by the low proliferation rate of human keratinocytes primary cultures [2]. One method to overcome this problem is the use of culture medium enriched with different biomolecules. Maslinic acid is a bioactive compound present in olive oil with multiple health benefits [3] and has been tested for different purposes. In this study, we evaluated the potential effect of this compound for improving keratinocyte culture protocols in skin, cornea, palate and oral mucosa tissue engineering.

Methods: Immortalized human skin keratinocytes were cultured with medium containing different concentrations of maslinic acid (1-80 µg/mL) for 24, 48 and 72 h. Cell viability and proliferation were assessed with WST-1, LIVE/DEAD (Fig. 1), flow cytometry and quantification of DNA in medium. Immunohistochemistry of KI-67 and gene expression analysis were performed for cells in presence of 5 µg/mL of maslinic acid, the concentration selected for the rest of the experiment. Then, the potential of 5 µg/mL of maslinic acid to improve the generation of keratinocyte cell cultures was evaluated for 21 days. Phase-contrast images were taken and cells were stained with hematoxylin-eosin for macroscopical images. Statistical analysis of results was carried out using Mann-Whitney test and Exact Test of Fisher.

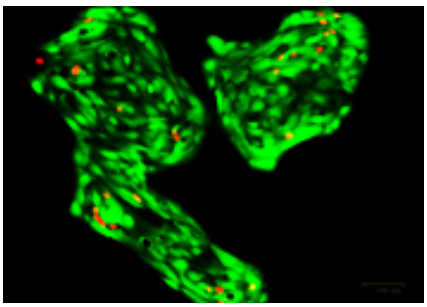


Fig. 1. Analysis of cell viability using LIVE/DEAD. Live cells are stained in green, whereas dead cells are shown in red.

Results: Low concentrations of maslinic acid maintain cell viability and only concentrations above 40 µg/mL promote cell death in immortalized human keratinocytes. Cell proliferation was improved in all concentrations but only 5 µg/mL of maslinic acid appeared to have a significant proliferation effect in the three times tested. This concentration also showed an increment in the proliferation rate of human keratinocyte cultures as compared to controls.

Discussion & conclusions: Our results are in agreement with previous studies which showed that high concentrations of maslinic acid dramatically reduce the number of viable cells. The selection of 5 µg/mL has the advantage of reducing the cytotoxic effect and stimulating cell proliferation. Although these findings may establish maslinic acid as a promising factor for enhancing keratinocyte growth in the manufacturing process of bioartificial skin, cornea, oral mucosa and palate, further research should be performed to clarify its potential effect.

This study was supported by projects PE-0395-2019 from the Consejería de Salud y Familias, Junta de Andalucía, Spain, and B-CTS-450-UGR20 (proyectos de I+D+i en el marco del Programa Operativo FEDER Andalucía 2014-2020), University of Granada and Consejería de Transformación Económica, Industria, Conocimiento y Universidades). Supported by the Spanish Plan Nacional de Investigación Cientifica, Desarrollo e Innovación Tecnológica (I+D+i) of the Spanish Ministry of Science and Innovation through grant FIS PI20/0317, FIS PI21/0980, ICI19-00024 (BIOCLEFT) and ICI21-00010 (NANOULCOR) from Instituto de Salud Carlos III, co-financed by the European Regional Development Fund (ERDF).

[1] Böttcher-Haberzeth S, et al. Burns. 2010;36(4):450-460
[2] Wang J, et al. Plos One. 2021; 16(7):e0254731
[3] Sánchez-Quesada C, et al. J. Agric. Food Chem. 2013;61(50):12173-82

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Melatonin-enriched keratinocyte media increases mitochondrial activity in cultured human epithelial cells

Ávila-Fernández P.[1], Guerra-Librero A.[2,3,4], García-García O.D.[1,4], González-Gallardo C.[4,5], Bermejo-Casares F.[1], Martín-Piedra M.A.[1,4], Alaminos M.[1,4], García J.M.[1,4], Garzón I.[1,4], Escames G.[2,3,4], and Chato-Astrain J.[1,4]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] CIBERfes, Ibs.Granada, Granada Spain; [3] Biomedical Research Center, Department of Physiology, Faculty of Medicine, Institute of Biotechnology, Technological Park of Health Sciences, University of Granada, Spain; [4] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [5] Division of Ophthalmology, University Hospital Virgen de las Nieves, Granada, Spain

Introduction: A rapid and efficient human epithelial cells expansion and proliferation is still one of the main unsolved problems in tissue engineering. In fact, a crucial step in the development of artificial skin, oral mucosa and cornea is to obtain sufficient quantities of epithelial cells in a short period of time. In order to increase cell growth and proliferation, epithelial culture medium is normally enriched with different growth factors and hormones, and new in-culture strategies such as encapsulated formulations, have been used to improve cell proliferation *ex vivo* [1]. However, new epithelial culture formulations are still needed. Recent studies focused on melatonin -an indolamine mainly synthesized by the pineal gland- showed promising results on the antioxidant activity of this agent, mainly due to its intrinsic ability of scavenge reactive oxygen species (ROS), which in turn would improve the efficiency of the mitochondrial electron transport chain [2]. In the present work, we evaluated the effect of melatonin on human epithelial cell cultures.
Methods: Preestablished cultures of normal human epithelial cells (CRL-4048 cells) were used. Cells were cultured with routine keratinocyte culture medium (KM) containing 10% FBS and growth factors, and kept under normal culture conditions (37ºC, 5% CO_2). Then, melatonin was added to the KM at different concentrations (1, 2 and 4 mM) and cell viability was assessed after 24, 48 and 72h of follow-up. KM medium was used as control. The mitochondrial activity was determined by the WST-1 metabolic assay, and cell counting by DAPI staining. Moreover, proliferation was determined by KI-67 immunofluorescent detection. Differences between were identified using the Kruskal-Wallis and Mann-Whitney statistical tests.
Results: Melatonin enrichment of KM showed an increase in the cell mitochondrial activity of normal human epithelial cells. As compared to the control group, 2mM Melatonin was able to significantly increase mitochondrial activity of cultured cells, with approximately 14% increase after 24h ($p=0.0147$). Nevertheless, cell counting and proliferation showed a significant decrease between melatonin-treated group and controls.
Discussion & conclusions: These preliminary results showed that melatonin-enriched culture media could contribute to enhance metabolic and mitochondrial activities of human epithelial cells kept in culture. Although results should be confirmed in the future, this increase in ATP synthesis could be related to an effect on epithelial phenotype differentiation, which could contribute to generate more biomimetic substitutes of the human skin, oral mucosa and cornea by tissue engineering.

Supported by the Spanish Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I) of the Spanish Ministry of Science and Innovation (Instituto de Salud Carlos III), grants FIS PI18/0331, FIS PI21/0980, FIS PI20/0317, ICI19/00024 (BIOCLEFT) and ICI21/00010 (NANOULCOR), and by grants PE-0395-2019 and PI-0442-2019 from the Consejería de Salud y Familias, Junta de Andalucía, Spain. Addition support was provided through grant B-CTS-450-UGR20 (proyectos de I+D+i en el marco del Programa Operativo FEDER Andalucía 2014-2020, University of Granada and Consejería de Transformación Económica, Industria, Conocimiento y Universidades), and cofinancing was provided from the European Regional Development Fund (ERDF) through the “Una manera de hacer Europa” program.

[1] Chato-Astrain J, et al. Biomedicines. 2021:9(11):1634
[2] Talib M.H. Molecules. 2018;23(3):518

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Generation of a biomimetic epithelial-stromal junction in bioengineered human tissues

Sánchez-Porras D.[1,2], Cases-Perera O.[3,4], Blanco-Elices C.[1,2], Ortiz-Arrabal O.[1,2], Fernández-Valadés R.[1,2,5], Alaminos M.[1,2], Sánchez-Quevedo M.C.[1,2], Garzón I.[1,2], Rodríguez I.A.[1,6] and Martín-Piedra M.A.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Doctoral program in Biomedicine, University of Granada, Spain; [4] Department of Plastic Surgery, University Hospital Virgen de las Nieves, Granada, Spain; [5] Division of Pediatric Surgery, University Hospital Virgen de las Nieves, Granada, Spain; [6] Cátedra "B" de Histología y Embriología, Facultad de Odontología, Universidad Nacional de Córdoba, República Argentina

Introduction: One of the main goals of tissue engineering is to biomimetically reproduce the structure of natural tissues and organs. In the case of the human skin and oral mucosa, generating the typical structure of the epithelial-stromal junction, including the rete ridges and chorial papillae, is very difficult to achieve using traditional biofabrication methods. Ridges and papillae play important roles in tissue physiology [1.2], especially to maintain and consolidate the dermo-epithelial junction and to for a niche able to host the epithelial stem cells. In this work, we have developed a novel biofabrication method able to generate rete ridges and chorial papillae in biomaterials used in tissue engineering.

Methods: First, we designed a pattern mold using 3D modelling and then were printed this mold using a FDM 3D printer and poly-lactic acid (PLA) as biomaterial with a resolution of 100 µm. Then, we generated different hydrogels based on 2% agarose and 0.1% agarose-fibrin biomaterials using the printed mold as a cast to generate a specific pattern during jellification. Once jellified, the molds were carefully removed to obtain a hydrogel with the specific patterns on its surface, that could be repopulated with epithelial cells. To evaluate the efficiency of the system, the surface of the hydrogels was then covered with melted type-VII agarose to resemble the epithelial layer.

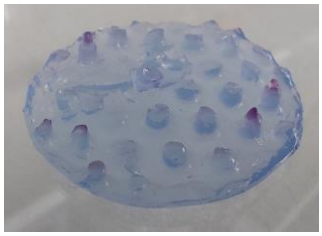


Fig. 1. Patterned hydrogel showing surface ridges.

Results: The application of the predesigned pattern mold to the different hydrogels allowed us to generate specific 3D structures resembling the epithelial rete ridges and chorial papillae. These structures were identified in the biomaterial as conical open cavities with a definite morphology that was partially analogue to the structures found at the epithelial-stromal interphase of the skin and oral mucosa dermo-epithelial junction. In addition, the use of low gelling agarose on tissues previously subjected to patterning with the printed mold resulted in the formation of different ridges whose height ranged between 1 and 2 mm (Figure 1).

Discussion & conclusions: Application of the novel biofabrication protocol described here allowed to generate complex bioartificial hydrogels with a epithelial-stromal junction containing specific structures resembling epithelial ridges and chorial papillae. These hydrogels could be used for the development of bioengineered substitutes of the human oral mucosa, skin or other structures containing complex dermo-epidermal structures such as the human palate. The presence of these specializations could improve epithelial nutrition and crosstalk between the epithelial and the stromal layers. Improvement of the epithelial-mesenchymal interaction could contribute to generate bioartificial tissues with more adequate differentiation patterns for use in tissue engineering.

Supported by the Spanish Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I) of the Spanish Ministry of Science and Innovation (Instituto de Salud Carlos III), grants FIS PI18/0331, FIS PI21/0980, and ICI19/00024 (BIOCLEFT), and by grants PE-0395-2019 and PI-0442-2019 from the Consejería de Salud y Familias, Junta de Andalucía, Spain. Addition support was provided through grant B-CTS-450-UGR20 (proyectos de I+D+i en el marco del Programa Operativo FEDER Andalucía 2014-2020, University of Granada and Consejería de Transformación Económica, Industria, Conocimiento y Universidades), and cofinancing was provided from the European Regional Development Fund (ERDF) through the "Una manera de hacer Europa" program.

[1] Wu T, et al. Cell Tissues Organs. 2013; 197(3):239-248
[2] Vela-Romera A, et al. Histochem Cell Biol. 2019;151(1):57-73

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Color variations among different shades, thickness and printing angle of 3D printing dental restorative polymer-based materials

Espinar C.[1,2] Tejada-Casado M.[1,2], Ruiz-López J.[1,2], Della Bona A.[3], Pulgar R.[2,4], Cardona J.[1,2], Ionescu A.M.[1,2] and Pérez M.M.[1,2]

[1] Biomaterials Optics Group, Department of Optics, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3]Postgraduate Program in Dentistry, Dental School, University of Passo Fundo, Passo Fundo, Brazil; [4]Department of Stomatology, Faculty of Dentistry, Colegio Máximo, Campus de Cartuja s/n. University of Granada. 18071. Granada. Spain

Objetives: Digital technology has leaded to a breakthrough in restorative dentistry [1]. This study explore the effect of shades, thickness and printing angle on the color of the recent 3D printing dental restorative polymer-based material

Materials and Methods: Specimens of 0.5, 1.0, 1.5 and 2.0mm thick (n=3) for A1, A2 and A3 shades of Freeprint® Temp (DETAX GmbH, Germany) were manufactured using a DLP printer (Asiga Max UV 385) with a 62microns pixel resolution and 0º and 90º printing angle. Spectral reflectance was measured and color coordinates were calculated on black background using a spectroradiometer PR-670, CIE D65 illuminant and the CIE 45°/0° geometry. CIEDE2000 color difference (ΔE_{00}) [2] between thicknesses and printing angles, for all shades, were evaluated using their respective 50:50% perceptibility and acceptability (PT and AT) thresholds [3]

Results and Discussion: CIELAB color coordinantes increase as the thickness increases with ΔE_{00} greater that acceptability thresholds AT (1.8 units) for all shades. Color differences between different printing angle were: $\Delta E_{00\ (0^o - 90^o)}$ A1= 2.6, 1.0, 2.5, 1.4; $\Delta E_{00\ (0^o - 90^o)}$ A2= 0.7, 0.6, 1.0, 3.0 and $\Delta E_{00\ (0^o - 90^o)}$ A3= 0.9, 0.4, 1.2, 2.1 for 2.0, 1.5,1.0 and 0.5mm, respectively. For the same thickness and printing angle, the shades showed color differences higher AT except to A1 and for shades of 0.5mm thick that PT< ΔE_{00} >AT was found. Thus, in general, color change due to difference in printing angle is visually perceptible and depend of the thickness and shade

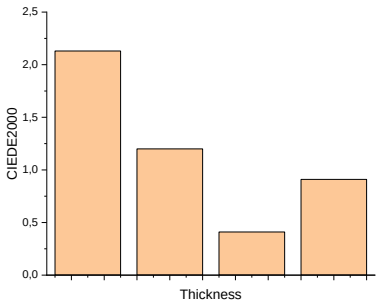


Fig. 1. Average color differences between printing angle 0º-90º samples

Conclusions: Color of 3D printing dental polymer-based material is affected by shade, thickness and printing angle. Such behavior must taken into account by dental technicians for a appropriate dental restoration.

Supported by P20-00200 and A.TEP.280.UGR18 from the Government of Andalusia, Spain, and PGC2018-101904-A-I00 from the Spanish Ministry of Science, Innovation and Universities, Spain

[1] Barazanchi A. et al. Additive technology:update on current materials and applications in dentistry.J Prosthodont. 2017;26:156-163.
[2] The International Commission on Illumination. CIE 015:2018 Colorimetry. 4th ed. Vienna, Austria; CIE Central Bureau; 2018
[3] Paravina RD. et al. Color Difference Thresholds in Dentistry. J Esthet Restor Dent. 2015;27(S1):S1–S9

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Study of the capacity of alignment and migration of neural and dental stem and progenitor cells cultured on nanopatterned polydopamine bioresorbable polymer scaffolds for cell therapy

Manero-Roig I.[1,2], Polo Y.[3], Pardo-Rodriguez B.[1], Romayor I.[1], Luzuriaga J.[1], Rubio-Emazabel L.[3], Eguizábal C.[4,5], Ibarretxe G.[1], Unda F.[1], Sarasua J.R.[1,3], Larrañaga A.*[1,6] and Pineda J.R.*[1,7]

[1] University of the Basque Country (UPV/EHU), Leioa, Spain; [2] Université de Bordeaux IINS-UMR5297, Bordeaux, France; [3] Polimerbio SL, Donostia-San Sebastian, Spain; [4] Cell Therapy, Stem Cells and Tissues Group, Biocruces Bizkaia Health Research Institute, Barakaldo, Spain; [5] Research Unit, Basque Centre for Blood Transfusion and Human Tissues, 48960 Galdakao, Bizkaia, Spain; [6] Polymat, Bilbao, Spain; [7]Achucarro Basque Center for Neuroscience Fundazioa, Leioa, Spain. * Corresponding authors

Introduction: Injuries in the central nervous system (CNS) and nerve lesions have a strong social impact in terms of high financial expenses, and quality of life of the affected patients. Cell therapies in the CNS are particularly complicated, because once neuronal connectivity is lost in a CNS region, there are no therapeutic strategies available to limit cellular dispersion or promote a guided neuronal cell migration to restore the preexisting circuitry. Moreover, there is a particularly acute shortage of sources of stem cells which can differentiate towards neural lineages to replace the lost neurons and glial cells, and the use of human brain neural stem and progenitor cells (NSCs) is very limited, for many practical reasons. Human dental pulp stem cells (hDPSCs) offer an excellent alternative of neural competent cells [1], which also secrete neurotrophins and anti-inflammatory factors, are resistant to hypoxic conditions, highly accessible, and without ethical concerns [2,3].
Methods: We fabricated scaffolds of polylactide and caprolactone based copolyesters nanopatterned with gratings of 300 nm line width to create a nanostructured bioresorbable polymer scaffold. We seeded either 1-3·10^5 hDPSCs or (control) murine NSCs (mNSCs) in 20 µL and determined they capacity of cellular adhesion without a need of laminin coating by cell videorecording for 24h, We assessed cellular alignment to nanogratings, migratory cell velocity, pausing time and distance travelled for both types of stem cells.
Results: Both mNSCs and hDPSCs cultured either on laminin or nanopatterned scaffolds attached to the substrate, instead of growing as floating neuro/ dentospheres. Contrary to laminin, nanopatterned scaffolds induced a progressive cellular elongation following the nanograting axis, generating chains of controlled cellular cluster migration along the nanogroove direction. Finally, immunohistochemistry analyses showed the expression of both neuronal and glial markers on both types of aligned human and murine cells, showing their neural commitment.
Discussion and conclusions: The nanostructured bioresorbable polymeric scaffold constitute a simple and scalable method to attach, align and guide the migration of both dental and neural stem and progenitor cells, offering an excellent alternative to promote a directed spatial cell growth and extension of cellular projections for future neuroregenerative therapies.

Funding: UPV/EHU (COLAB19/03; GIU20/050; GIU16/66; UFI11/44; IKERTU-2020.0155) GV/EJ (Dep. Salud 2021333012; GV-GIC15/52; IT-927-16; IT831-13;Hazitek ZE-2019/00012-IMABI & ELKARTEK KK-2019/00093), MICINN (PID2019-104766RB-C21), MINECO (RYC-2013-13450). Polimerbio and Y. P. have a Bikaintek PhD grant (20-AF-W2-2018-00001). B. Pardo-Rodriguez has a Predoctoral for non-doctoral research staff from de Basque Government (Ref. 2021_1_0112). I. Manero have a UPV/EHU mod-III grant (PIFBUR21/05).

[1] Gronthos, Mankani, Brahim, Robey & Shi (2000). Proc Natl Acad Sci U S A 97(25):13625-30.
[2] Yoshida, S., et al. (2020). Biology (Basel) 9(7), 160.
[3] Luzuriaga J, Polo, Y, et al. (2021). Int. J. Mol. Sci 22(7), 3546.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Generation of fibrin-based electrospun nanofibrous scaffolds for oral mucosa applications in tissue engineering

Blanco-Elices C.[1,2,3,4], Campos F.[2,3], Sola M.[2,3], Fernández-Valadés R.[2,3,4], de la Cueva-Batanero P.[2], Alaminos M.[2,3], García J.M.[2,3], Martín-Piedra M.A.[2,3], Garzón I.[2,3] and Liu X.[5]

[1] Doctoral program in Biomedicine, University of Granada, Spain; [2] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [3] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [4] Division of Pediatric Surgery, University Hospital Virgen de las Nieves, Granada, Spain: [5] Department of Biomedical Sciences, College of Dentistry, Texas A&M University, Dallas, TX, USA

Introduction: Tissue engineering is a promising strategy to regenerate bioartificial human oral mucosa substitutes for the treatment of patients who have oral mucosa damages. Several synthetic and natural biomaterials as well as different design strategies have been proposed for fabricating tissue engineering oral mucosa scaffolds [1]. Among them, naturally derived polymers such as fibrin and collagen have the advantages of excellent biocompatibility, cell-specific interactions, and hydrophilicity. In this regard, the aim of the present study was to fabricate a fibrinogen-based electrospun nanofibrous scaffold using gelatin as an “electrospinning-driving” polymer.

Methods: Fibrin is a polypeptide consisting of the plasma components fibrinogen and thrombin. Fibrinogen was prepared in distilled water and mixed with a solution consisting of of 17% of gelatin (type B), 25% of acetic acid, 15% of ethyl acetate and 50% of 1, 1, 1, 3, 3, 3-Hexafluoro-iso propanol (HFIP). The solution was taken in 5 ml syringe and connected to a delivery device. The delivery device dispensed the solution at a predetermined flow rate of 1 ml/h. A high voltage power supply was used to apply a voltage 22 kV to a 22-gauge blunt end needle fixed to a shaped connector attached with the syringe containing the solution. The solution was electrospun on an aluminum foil attached to a grounded mandrel placed at 10-15 cm from the needle tip (Figure 1). The scaffold was removed from the mandrel soon after electrospinning and crosslinked. Morphological characterization of the electrospun scaffold was performed using scanning electron microscopy (SEM).

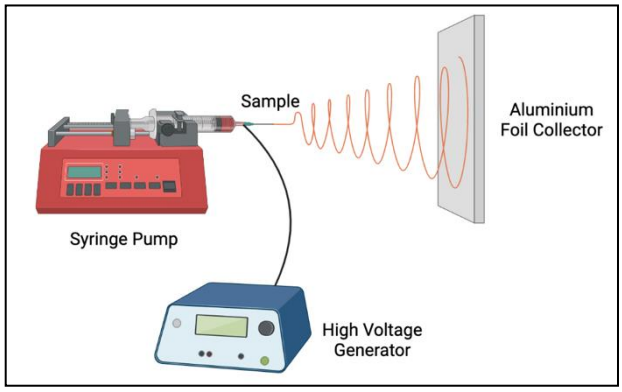


Fig. 1. Schematic representation of the electrospinning process.

Results: Electrospinning of natural polymers including fibrinogen requires highly volatile solvents such as HFIP. However, HFIP and other harsh organic solvents are toxic and may affect the biological activity of the natural fibrin scaffold. This is why we used a low concentration of this organic solvent. In addition, previous studies revealed the low solubility of fibrinogen in HFIP. Due to this fact, gelatin solution was used as an “electrospinning-driving” polymer in the present study. Several parameters, such as flow rate and electric field, affected the electrospinning process. However, the scanning electron micrograph of the electrospun scaffold displayed a homogenous nanofibrous structure.

Discussion & conclusions: This work reports a novel electrospinning method for the fabrication of nano fibrous fibrin-based scaffold. However, further *in vitro* and *in vivo* biocompatibility analysis are required to determine the potential use of this promising new scaffold in oral mucosa tissue engineering protocols.

This study was partially supported by the Spanish Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I) of the Spanish Ministry of Science and Innovation (Instituto de Salud Carlos III), grants FIS PI18/331, FIS PI21/00980 and ICI19/00024 (BIOCLEFT), and by grant CSyF PI-0442-2019 by Consejería de Salud y Familias, Junta de Andalucía, Spain. Co-funded by FEDER funds, European Union.

[1] Perumcherry SR, et al. Tissue Eng Part C Methods. 2011;17(11):1121-30

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Building silk-fibroin 3D hydrogels with enzymatically cross-linked vitronectin to study neuroblastoma aggressiveness

Vieco-Martí I.[1,2], Monferrer E[1,2], López-Carrasco A.[1,2], Granados-Aparici S.[1], Navarro S.[1,2] and Noguera R.[1,2]

[1] Department of Pathology, Medical School, INCLIVA Biomedical Health Research Institute, University of Valencia, Valencia, Spain; [2] Low Prevalence Tumors, Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Instituto de Salud Carlos III, Madrid, Spain.

Introduction: Vitronectin (VN) is a glycoprotein involved in biological processes such as cell growth, angiogenesis and metastasis in different tumors. The high-risk neuroblastoma (HR-NB) tumors have a huge expression of VN. The presence of VN may confer mechanical properties which increase HR-NB aggressiveness [1]. To further study the VN role in the HR-NB, the building of a 3D in-vitro platform is required. Silk-fibroin (SF) is an easy tunable biomaterial which enable the building of hydrogels with enzymatically-linked molecules. Furthermore, the degradation products of the SF are not toxic for the cells. The aim of this study is to build a SF 3D model with different amounts of VN to study its role in the aggressiveness of the neuroblastoma.

Methods: SF 3D models are based on previous works [2,3]. Briefly, SF (Sigma Aldrich) was mixed with of gelatin-tyramine (G-TA, Sigma Aldrich) in non-complemented IMDM cell culture medium to obtain a 4% w/v solutions. We tested two proportions of G-TA : SF, 25_75 and 50_50. Furthermore, we made duplicates in which we added VN (PrepoTech) at a final concentration of 0.4mg/mL. HR-NB cell line SK-N-BE(2) was resuspended in the pre-hydrogel solution, at a final density of 2 million cells/mL. To start the polymerization reaction, we added horse radish peroxidase (20 U/mL) and hydrogen peroxide (0.01%). Aliquots of 60 µL were placed in a 24-wheel plate and kept at 37ºC for 1 hour. Afterwards, 1.5 mL of complemented IMDM cell culture medium was added, and changed every two days. We kept the 3D models for 3, 7, 14 and 21 days. At those time-points the hydrogels were paraffin embedded, and 3 micron slices were hematoxylin-eosin (H&E) stained. A VN immunostaing was done to check its attachment to the hydrogel. A total of 135 H&E slices were digitalized with Ventana Scanner (Roche) and analyzed in QuPath. We measured the size of the cells clusters and computed de density of the clusters, defined as number of clusters per unit of hydrogel area. All the data and graphics were processed with R.

Results: The immunohistochemistry shows that the VN is uniformly attached to the VN-containing hydrogels, whereas no staining is observed in nonVN-containing hydrogels. Regarding time points, the cluster size in all conditions reached the maximum median size at day-14. However, the density of the clusters reached the maximum median at day 21 in all conditions. Regarding VN, at day-21 the 25_75 VN-containing hydrogels had a lower size than its counterpart without VN, whereas in 50Ç_50 hydrogels this difference it is not evident. The presence of VN at 25_75 models trends to increase the density of clusters, whereas this tendency it is not evident at 50_50 models.

Discussion & conclusions: We successfully build silk-fibroin 3D hydrogels with enzymatically cross-linked VN. We observed different behaviors of HR-NB cells in different conditions. Those 3D models are suitable to perform further studies to reveal the role of the VN in the aggressiveness of the neuroblastoma. It has not scape our notice the potential use of those hydrogels as mechano-drug testing platform.

Supported by: Spanish University Ministry (FPU20/05344); ISCIII PI20/01107; CIBERONC (CB16/12/00484)

[1] Burgos-Panadero R., Noguera I., Cañete A., Navarro S. and Noguera R. (2019). Vitronectin as a molecular player of the tumor microenvironment in neuroblastoma. BMC cancer, 19(1), 479.
[2] Sakai S., Hirose K., Taguchi K., Ogushi Y. and Kawakami K. (2009). An injectable, in situ enzymatically gellable, gelatin derivative for drug delivery and tissue engineering. Biomaterials, 30(20), 3371–3377.
[3] Hasturk O., Jordan K. E., Choi J. and Kaplan D. L. (2020). Enzymatically crosslinked silk and silk-gelatin hydrogels with tunable gelation kinetics, mechanical properties and bioactivity for cell culture and encapsulation. Biomaterials, 232, 119720.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Fibrin/agarose-based chondrogenic constructs for articular cartilage repair. A preliminary study

Sánchez-Porras D.[1,2], Losilla-Martínez J.M.[3], Cases-Perera O.[3], Ortiz-Arrabal O.[1,2], Sola M.[1,2], de la Cueva-Batanero P.[1], Rodríguez I.A.[1,4], Campos A.[1,2], Campos F.[1,2] and Carriel V.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Servicio de Cirugía Plástica y Reparadora, Hospital Universitario Virgen de las Nieves, Granada, Spain; [4] Cátedra "B" de Histología y Embriología, Facultad de Odontología, Universidad Nacional de Córdoba, República Argentina

Introduction: The articular cartilage can be affected by traumatic injuries, degenerative processes or autoimmune conditions. These highly prevalent conditions frequently cause pain, loss of joints mobility and a decrease of the quality of life in affected patients. Unfortunately, current treatments do not achieve optimal regenerative and functional recovery results. One of the most effective treatments is based on the use of cultured autologous articular chondrocytes. However, in most cases, pathological chondrocytes have both a low proliferation rate and poor capability to synthetize extracellular matrix (ECM) components, and the search of alternative cell sources for cartilage tissue engineering is in need. In this context, several models of bioengineered cartilage have been developed by tissue engineering [1]. One of the biomaterials showing promising results in cartilage tissue engineering is fibrin/agarose (FA) [2]. The aim of this preliminary study was to determine the potential usefulness of FA hydrogels containing two different cell sources: elastic chondrocytes (EC) and adipose-derived mesenchymal stem cells (ADSC) for potential use in articular cartilage repair.
Methods: Defects of 5-mm of diameter and 2-mm of depth were generated in the femoral condyles of New Zealand laboratory rabbits. The defects were then repaired with cartilage substitutes consisting of EC immersed within a FA scaffold (FA-EC) or with cartilage substitutes containing a combination of EC and ADSC immersed in a FA scaffold (FA-EC/ADSC). Unrepaired lesions were used as negative controls. Cartilage regeneration was analyzed by macroscopic and histological analyses after 12 weeks of in vivo follow-up (n=3 each group).
Results: Macroscopy revealed a newly-formed cartilage covering the defects in all treated animals. The FA-EC group showed a clear and complete repair of the defects, with a similar composition to the native control. In contrast, FA-EC/ADSC regeneration was poor in terms of morphology and ability to fill the defects. Histological analysis of FA-EC showed similar results to the control, with a homogeneous ECM and chondrocyte-like cell morphology, with a smooth appearance in the superficial zone and a visible tide-mark in some areas. In contrast, the FA-EC/ADSC group showed irregular regeneration, with discontinuous ECM throughout the defect and heterogeneous histological pattern.
Discussion: Our preliminary results suggest the potential usefulness of FA-based hydrogels in articular cartilage repair. Regarding the cells used, EC offered more positive regeneration and resulted to be a promising cell source when combined with FA hydrogels to repair articular hyaline cartilage defects, with good macroscopic and histological results. In contrast, the use of FA with EC/ADSC did not show optimal results. Further studies are still needed to elucidate the cellular and molecular mechanisms involved in the regeneration process of articular cartilage promoted by implanted FA-EC.

This study was supported by grant PI-0257-2017 from Consejería de Salud y Familias, Junta de Andalucía, Spain and CTS-115 (Tissue Engineering Group).

[1] Sánchez-Porras et. al. (2021) Biomedicines. Mar 12;9(3):292
[2] Garcia-Martinez L, et al. (2017) Histochem Cell Biol. 147(1):83-95

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Tissue engineering for the regeneration of articular cartilage injuries in a porcine model: Morphological evaluation with the ICRS II scale

Aguilar A.[1,2,3], Milián L.[1,2], Zurriaga J.[1], Ródenas J.[4], Mata M.[1,2], Martín de Llano J.J.[1,2], Gallego G.[4,5], Gómez Ribelles J.L.[4,5], Carda C.[1,2,5] and Sancho-Tello M.[1,2]

[1]Department of Pathology, Universitat de València, Spain; [2]INCLIVA Biomedical Research Institute, Valencia, Spain; [3]Hospital IMED Valencia, Spain; [4]Centre for Biomaterials and Tissue Engineering (CBIT), Universitat Politècnica de València, Spain; [5]Biomedical Research Network Centre on Bioengineering, Biomaterials and Nanomedicine, Spain

Background: Articular cartilage injuries are a growing cause of disability in the world population. It has excellent biomechanical properties but little regeneration capacity, and its repair process usually causes a fibrocartilage scar. Tissue engineering using scaffolds is an interesting option, as they provide adequate support for pluripotential cells to migrate and differentiate into chondrocytes. There are multiple scaffold conformation designs, synthesized from multiple biomaterials. In this study we used microspheres that we have previously used in a rabbit model with promising results.
Methods: A porcine animal model was designed to evaluate articular cartilage regeneration using synthesized scaffolds. Microspheres of two different biomaterials were synthesized: polylactic acid (PLA) and platelet-rich plasma (PRP) obtained from autologous blood. A PLA membrane was also synthesized to cover the injury site, thus preventing migration of the microspheres. A 6mm diameter, full-thickness cartilage injury was created in the medial condyles of the knees in all groups. Treatment groups (n=8 per group) were: 1) negative control group, only received PLA membrane; 2) Novocart (Braun®) control group, treatment already approved for articular cartilage injuries; and 3) experimental group with PLA+PRP microspheres and PLA membrane. After 10 months, knee samples were obtained and processed. Scale II of the International Cartilage Research Society (ICRS) [1] was used to assess the quality of regenerated tissue.

Results: The results obtained are shown in Table 1:

Table 1. ICRS II scale in the experimental groups

Parameters studied	Negative control	Novocart	PLA+PRP microspheres
Tissue morphology	46.87	60.71	59.37
Cell morphology	56.25	57.14	53.12
Chondrocyte clustering	81.25	78.57	78.12
Surface architecture	68.75	96.42	87.50
Basal integration	81.25	92.85	93.75
Formation of a tidemark	0.00	12.50	0.00
Subchondral bone abnormalities	93.75	82.14	93.75
Inflammation	75.00	57.14	59.37
Abnormal calcification/ossification	100.00	100.00	100.00
Vascularization	62.50	89.28	84.37
Surface/superficial assessment	62.50	75.00	68.75
Mid/deep zones assessment	43.75	39.28	37.50
Overall assessment	**70.11**	**70.75**	**72.65**

Furthermore, no membrane debris was observed in any sample, while biomaterial remaining was observed in 2 samples from the Novocart group. Native normal cartilage has a score of 100.
Discussion and Conclusions: Our PLA+PRP microspheres scaffold showed similar histological results compared to the currently stablished Novocart, supporting its future application in humans. The absence of microspheres debris in the samples indicates that the resorption rate is adequate to provide a good support for the migrating cells while not lasting too long in the tissue.

Supported by the Spanish Ministry of Science and Innovation (Grant PDC2021-121658-C22).

[1] Mainil-Varlet P., Van Damme B., Nesic D., Knutsen G., Kandel R. and Roberts S. (2010). A new Histology scoring system for the assessment of the quality of human cartilage repair: ICRS II. Am J. Sports Med. 38(5): 880-90

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Generation of bioartificial scleral substitutes for the microsurgical repair of the external eye layer

Vizcaíno-López G.[1,2], González-Gallardo C.[3,4], Campos F.[1,3], Carriel V.[1,3], Sánchez-Montesinos I.[3,5] and Campos A.[1,3]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Department of Histology, Autonomous University of Santo Domingo, Dominican Republic; [3] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [4] Division of Ophthalmology, University Hospital Virgen de las Nieves, Granada, Spain; [5] Department of Human Anatomy and Embryology, University of Granada, Spain

Introduction: Tissue engineering techniques offer the possibility of creating new substitutes for the repair of structural defects of the eyeball. Therefore, our goal includes generate and characterize a new biomimetic model of bio-artificial sclera based on the use of nanostructured fibrin-agarose biomaterials for surgical repair of the ocular wall.

Methods: In this study, 18 male New Zealand rabbits were used, which were distributed into 6 groups. Nanostructurated fibrin-agarose hydrogel (NFAH) biomaterials were prepared, combining them with chemical cross-linking with 0.5% glutaraldehyde or 0.1% genipin. A partial surgical resection of the sclera of the rabbits was performed. The graft was evaluated at 40 days, at which time the rabbits were sacrificed. The macroscopic, histological and histochemical morphological characteristics were analyzed. Each group was separated according to the graft placed as follows: NFAH, NFAH-GP, NFAH-GA and cadaveric graft (C-CTR). In addition, the lesion without repair was allowed to evolve as a negative control (N-CTR). The healthy right eye of each animal was used as a healthy control (H-CTR).

Results: The biocompatibility and biodegradation characteristics of NFAH were better than the contrasted materials ($p<0.005$). At the evaluation at 40 days, the inflammatory response was greater in the biomaterials with chemical cross-linking, being null for the C-CTR. The most similar regenerated tissue to H-CTR was NFAH. Regarding graft integration, the C-CTR group presented incomplete graft integration. Not repairing the defect (N-CTR) causes a 50% decrease in final scleral thickness ($p=0.000$) (see the extensive results in reference 4).

Discussion & conclusions: The use of biomaterials is a promising alternative for scleral reconstruction. The results were especially promising after the use of NFAH. This biomaterial showed a high degree of biocompatibility, biodegradation and tissue regeneration 40 days after surgery. Finally, this study suggests that NFAH could be an ideal biomaterial for the repair of scleral defects. However, future studies will determine the potential clinical translation of this product in reconstructive surgery of the eyeball.

Supported by CTS-115 (Tissue Engineering Group) and by Grant CS PI-0400-2016, Consejería de Salud y Familias, Regional Ministry of Health, Junta de Andalucía, Spain.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Production of relevant soluble ECM components by tissue decellularization for use in cornea tissue engineering

Campos F.[1,2], García-García O.D.[1,2], González-Gallardo C.[2,3], Ávila-Fernández P.[1], Bermejo-Casares F.[1], de la Cueva-Batanero P.[1], Garzón I.[1,2], García J.M.[1,2], Campos A.[1,2] and Alaminos M.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Division of Ophthalmology, University Hospital Virgen de las Nieves, Granada, Spain

Introduction: Several models of bioartificial human corneas have been generated by tissue engineering using a variety of materials. One of the models showing promising results in patients is the NANOULCOR model based on a combination of epithelial and stromal human cornea cells and fibrin-agarose (FA) biomaterials [1,2]. Although FA demonstrated to be highly biocompatible, its histological structure and biochemical composition differs from those of the native human cornea extracellular matrix (ECM). In contrast, decellularization allows an efficient generation of biomaterials containing most components of the native organ [3], although decellularized biomaterials are typically very difficult to repopulate with human cells. In the present work, we propose a novel biofabrication method based on the combination of FA hydrogels and relevant components of the cornea ECM generated by decellularization of native organs.

Methods: On the one hand, we generated a cornea substitute corresponding to the NANOULCOR model by combining cultured cells with FA biomaterials. On the other hand, we obtained decellularized porcine cornea scaffolds by applying a method previously developed by our group for cornea and limbus decellularization. This method was based on a combination of osmotic shock methods and SDS detergent treatment of porcine corneas. Then, the ECM composition was evaluated in both models by using histochemical and immunohistochemical methods.

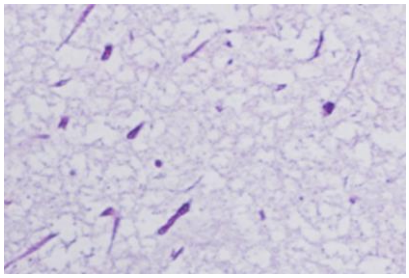


Fig. 1. Analysis of collagen fibers in the NANOULCOR model using picrosirius red

Results: First, our histological of the NANOULCOR corneas showed that these cornea substitutes consisted of a dense ECM containing stromal keratocytes and an epithelial substitute on top. Then, we found that the ECM of these bioartificial corneas was especially enriched in proteoglycans, and contained very low amounts of collagen fibers (Figure 1). In contrast, decellularized corneas contained high amounts of fibrillar components of the ECM, especially collagen, and less proteoglycans.

Discussion & conclusions: These results confirm that, despite its high biocompatibility, the ECM composition of the NANOULCOR model differs from that of the native cornea, especially regarding the presence of collagen fibers. The high abundance of these components in decellularized scaffolds suggest that a combination of both materials could provide an efficient functionalization of the FA biomaterial currently used in the NANOULCOR model. Future studies should determine the best combinatorial method to obtain mixed biomaterials with improved biological potential in tissue engineering of the human cornea.

Supported by the Spanish Plan Nacional de Investigación Cientifica, Desarrollo e Innovación Tecnológica (I+D+i) of the Spanish Ministry of Science and Innovation through grant FIS PI20/0317 and ICI21-00010 (NANOULCOR) from Instituto de Salud Carlos III, co-financed by the European Regional Development Fund (ERDF). Supported by grant CSyF PI-0086-2020 from the Consejería de Salud y Familias, Junta de Andalucía, Spain, and grant B-CTS-504-UGR20 (proyectos de I+D+i en el marco del Programa Operativo FEDER Andalucía 2014-2020, University of Granada and Consejería de Transformación Económica, Industria, Conocimiento y Universidades). Cofinanced by the European Regional Development Fund (ERDF) through the “Una manera de hacer Europa” program.

[1] Rico-Sánchez L, et al. J Tissue Eng Regen Med. 2019;13(12):2142-2154
[2] González-Andrades M, et al. BMJ Open. 2017;7(9):e016487
[3] Talaei-Khozani T, Yaghoubi A. Transpl Immunol. 2022 Jun 3:101640

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Functionalization of decellularized corneal limbi may improve the efficiency of tissue recellularization protocols

Sánchez-Porras D.[1,2], González-Gallardo C.[2,3], Ávila-Fernández P.[1], Sola M.[1,2], Fernández-Valadés R.[1,2,4], Oyonarte S.[1,2,5], Crespo P.V.[1,2], Campos A.[1,2], Alaminos M.[1,2] and Campos F.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Division of Ophthalmology, University Hospital Virgen de las Nieves, Granada, Spain; [4] Division of Pediatric Surgery, University Hospital Virgen de las Nieves, Granada, Spain; [5] Network for Transfusional Medicine, Cells and Tissues, Granada, Spain

Introduction: Reproduction in the laboratory of complex anatomical structures such as the sclerocorneal limbus is challenging, especially when synthesis hydrogels are used as scaffold biomaterials. However, decellularized native tissues offer the possibility to obtain orthotypical three-dimensional structures resembling the sclerocorneal limbus and its associated structures such as the limbal palisades of Vogt [1]. Although the decellularization method has been previously described, very few works have been able to recellularize these scaffolds to generate a biological substitute of the sclerocorneal limbus. In fact, recellularization is limited by the loss of adhesion molecules on the surface of decellularized tissues, what makes recellularization very difficult [2]. In this work, we evaluated the potential of a functionalization protocol to promote cell adhesion and recellularization of decellularized sclerocorneal limbi.

Methods: Decellularized porcine sclerocorneal limbi were generated using 0.1% SDS. Then, decellularised limbi were preconditioned by sequential washing in PBS and incubation in fetal bovine serum (FBS) for increasing incubation times. Then, functionalized limbi were recellularized with precultured SYRC corneal epithelial cells (CEC), and cell adhesion was analyzed after 7, 14 and 21 days of *ex vivo* culture. Epithelial phenotype was analyzed by pancytokeratin immunohistochemistry, and the synthesis of relevant components of the tissue extracellular matrix (ECM) was analyzed by histochemistry.

Results: Decellularized porcine sclerocorneal limbi functionalized with FBS showed a good attachment of epithelial cells on surface. Cells were able to stratify and differentiate with time, and a sequential expression of collagen fibers (as determined by picrosirius red staining) and proteoglycans (as determined by alcian blue). Cultured epithelial cells expressed pancytokeratin from day 7 onward.

Discussion & conclusions: Results demonstrated that functionalization with FBS proteins is able to modify the surface of decellularize limbi to allow cell adhesion. Cornea epithelial cells attached to the scaffold were able to express relevant markers of epithelia and contributed to remodeling the ECM of decellularized limbi. In conclusion, this method could be used to promote recellularization of decellularized xenograft used in tissue engineering.

Supported by grant CSyF PI-0086-2020 from the Consejería de Salud y Familias, Junta de Andalucía, Spain, and grant B-CTS-504-UGR20 (proyectos de I+D+i en el marco del Programa Operativo FEDER Andalucía 2014-2020, University of Granada and Consejería de Transformación Económica, Industria, Conocimiento y Universidades). Cofinanced by the European Regional Development Fund (ERDF) through the “Una manera de hacer Europa” program. Supported by the Spanish Plan Nacional de Investigación Cientifica, Desarrollo e Innovación Tecnológica (I+D+i) of the Spanish Ministry of Science and Innovation through grant FIS PI20/0317 and ICI21-00010 (NANOULCOR) from Instituto de Salud Carlos III, co-financed by the European Regional Development Fund (ERDF).

[1] Sánchez-Porras D, et al. Pharmaceutics. 2021;13:1718
[2] Tang-Quan KR, et al. Cell Mol Bioeng. 2020;13(6):633-645

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Generation of limbal crypts by using a novel biofabrication method on bioengineered sclerocorneal limbi

Campos F.[1,2], Ávila-Fernández P.[1], Ortiz-Arrabal O.[1,2], Martín-Piedra M.A.[1,2], Blanco-Elices C.[1,2], Sola M.[1,2], Sánchez-Quevedo M.C.[1,2], Oyonarte S.[1,2], Campos A.[1,2] and Alaminos M.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain

Introduction: The sclerocorneal limbus is a very complex organ hosting the limbal stem cells. Its fine three-dimensional structure contains specialized crypts that penetrate within the stromal tissue to generate the Vogt palisades whose function is crucial for a proper homeostasis of the limbal stem cells. Although several models of human bioartificial corneas have been generated in the laboratory [1,2], biomimetically reproducing in the laboratory the structure of the sclerocorneal limbus has not been achieved to the date. Here, we have developed a novel biofabrication method to promote the generation of crypts in biomaterials used in limbal tissue engineering.

Material and methods: We first obtained acellular limbal scaffolds by applying decellularization protocols to porcine corneas. Then, we generated a specific crypt device using a 3D printer using PLA biomaterials. This device consisted in a mold in which an elliptic pattern was designed, and 11 needles with a diameter of 500µm were inserted on this pattern, with an inclination of approximately 30º towards the exterior of the curve in order to reproduce the orientation of the native limbal crypts (Figure 1). This device was subjected to autoclave sterilization and used to fabricate specific patterns on the limbal scaffolds previously generated by decellularizing porcine xenografts, and cultured epithelial cells were then used to recellularize these xenografts. Histological analysis were used to identify the efficiency of the system.

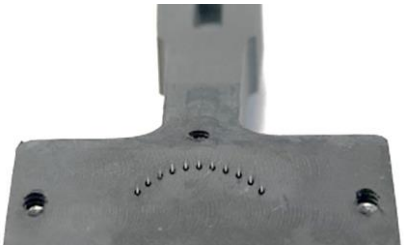


Fig. 1. Crypt device showing the needles inserted on the pre-designed pattern.

Results: Application of the crypt device was able to generate specific patterns on the decellularized limbal scaffolds. Cornea epithelial cells demonstrated to be able to repopulate the patterns and grow and develop within the generated crypts. Interestingly, cells found on these patterns showed positive expression of cytokeratins, suggesting a proper differentiation of these cells on the limbal xenografts.

Discussion & conclusions: The use of this crypt device showed to be useful to generate specific patterns on xenograft biomaterials. This pattern was able to host cells used to recellularize the scaffolds, and these cells were able to properly differentiate within the pattern. In conclusion, the crypt device could contribute to a more efficient and biomimetic biofabrication process of bioartificial sclerocorneal limbi by tissue engineering.

Supported by grant CSyF PI-0086-2020 from the Consejería de Salud y Familias, Junta de Andalucía, Spain, and grant B-CTS-504-UGR20 (proyectos de I+D+i en el marco del Programa Operativo FEDER Andalucía 2014-2020, University of Granada and Consejería de Transformación Económica, Industria, Conocimiento y Universidades). Cofinanced by the European Regional Development Fund (ERDF) through the “Una manera de hacer Europa” program.

[1] Rico-Sánchez L, et al. J Tissue Eng Regen Med. 2019;13(12):2142-2154
[2] Khosravimelal S, et al. Small. 2021;17(30):e2006335

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Zn-Substituted monetite based material induces high bone regeneration: a comparative study in a vertical augmentation model

Benito-Garzón L.[1], Díaz-Güemes I.[2], Enciso S.[2] and Padilla S.[3]

[1] Departamento de Anatomía e Histología Humanas, Facultad de Medicina, Universidad de Salamanca, Salamanca, Spain; [2] Instituto Centro de Cirugía de Mínima Invasión Jesús Usón, Cáceres, Spain; [3] Departamento de Química en Ciencias Farmacéuticas, Facultad de Farmacia, Universidad Complutense de Madrid, Spain.

Introduction: Dental implants can only be implanted if there is enough healthy bone to stabilize them adequately. If not, bone augmentation is mandatory before implant placement. However, vertical bone augmentation is a major surgical clinic challenge and is not always achieved. For bone volume augmentation is necessary to use a fill material, like autogenous bone, allograft, xenograft and synthetic bone grafts. Autogenous bone grafting is the most frequently used, despite its significant disadvantages. Allogenic bone from donor patients or xenograft materials, such as organic bovine bone (ABB), has been used as an alternative to autologous bone. However, non-resorbable or very slowly resorbable materials compromise the ultimate goal of complete regeneration of bone defects and may interfere with the integration of endosseous implants. More resorbable synthetic calcium phosphates, such as β-tricalcium phosphate (β -TCP) are also used. In this study a material (MSi) composed of Zn-substituted monetite, amorphous calcium phosphate, TCP and silica gel, with different resorption rate, was developed [1]. The present work is focused on the evaluation of the vertical bone augmentation and bone regeneration capacity of a MSi compared with ABB and β -TCP.

Methods: An osteoconductive model in titanium cylinders implanted in rabbit calvaria after 10 weeks was conducted. Their bone regeneration capacity and replacement by new osseous tissue were evaluated. Micro Computer Aided Tomography (Micro-CAT) was carried out before the histological processing to determine the bone volume formed within the rings and histomorphometrical analysis. Samples were subjected to nondecalcified ground sectioning. Stevenel's Blue stain was used. Some sections were photographed using backscatter electron microscopy (BS-SEM).

Results: ABB granulates filled over 90 vol% of cylinder. New bone vertically grew up to 55 vol% of the cylinder height. Newly formed bone area was 23% and osteointegrated ABB granules occupied 22%. New trabecular bone was formed in a thin and irregular, acquired the same sharp morphology as the granules. ABB granules did not show significant morphological changes after implantation. Non-integrated granules appeared surrounded by connective tissue with no signs of invasion within granules. β-TCP granulates maintained volume only up to 80%. New bone vertically grew up to 41 vol%. New bone area was 24% and osteointegrated β-TCP granulates 18%. Trabecular bone formation mainly occurred near the calvaria. Newly formed bone presented very thin trabeculae. Non-osteointegrated particles surrounded by connective tissue were observed. MSi granulates filled over 91 vol%. New bone vertically grew up to 58 vol%. New formed bone area occupied 39% and osteointegrated particles were 15%. New trabecular bone presented osteointegrated material and occupied almost the top of the cylinder. MSi osteointegrated particles presented bone formation and cell bone colonization inside them.

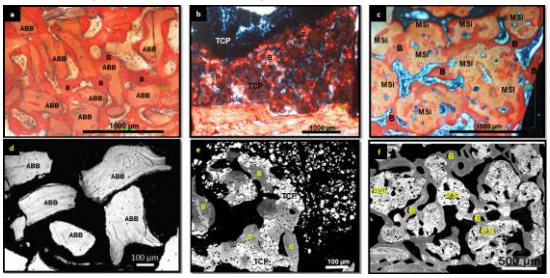


Fig. 1. Histological (a-c) and BS-SEM images (d-f). ABB (a and d), β -TCP (b and e) and MSi (c and f). (B: bone).

Discussion & conclusions: The evaluated materials were capable of vertical bone augmentation but with a very different behaviour. ABB maintained a high volume of the defect filled as it remains in the site osteointegrated without resorption. β-TCP lost its granular structure and appeared disintegrated in small particles because of its high resorption rate. β-TCP showed a significant lowest volume augmentation and a higher amount of non-osteointegrated material. On the contrary, MSi achieved the highest amount of new bone and remaining particles were osteointegrated. MSi composition allowed a different reabsorption rate which favoured bone regeneration.

Supported by Programa Nacional INNPACTO (ECC/1345/2012) (IPT-2012-0560-010000) and Subprograma RETOS-COLABORACIÓN (RTC-2014-1731-1).

[1] Padilla S, et al. Biomater Sc. Eng 2020, 6, 3440–3453

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Morphological and cytoskeletal changes induced in human osteoblasts by cell-biomaterial interactions with hybrid silica/chitosan aerogels

Pérez-Moreno A.[4], Reyes-Peces M.V.[4,7], Montesinos R.[1,2], Mesa-Díaz M.M.[6], de la Orden E.[1,2,3], García Palomeque J.[1,2], García Gomez N.[1], Vilches-Pérez J.I.[1,2,3], de la Rosa-Fox N.[4,5,7], Piñero-de los Ríos M.[4,5,7] and Salido-Peracaula M.[1,2,3]

[1]Department of Histology, School of Medicine, SCIB, University of Cádiz, Spain. [2] CTS253 Group, Andalusian Research Program (PAIDI). [3] Cadiz Biomedical Research Institute (INIBICA) Group EM26, Tissue Engineering. [4]Dept. of Condensed Matter Physical University of Cádiz, Spain. [5] TEP115 Group PAIDI.[6]Dept. of Chemical Engineering and Food Technologies, University of Cádiz, Spain. [7] Electron Microscopy and Materials Institute UCA (IMEYMAT).

Introduction: Bone tissue has the capacity to regenerate in response to several injuries in a process largely mediated by osteoblasts. During osteogenesis and regeneration processes, osteoblasts differentiate into osteocytes, which represent the final differentiation step in the osteoblastic lineage. In the process, osteoblasts lose a large part of their cell organelles but gain long, thin, and branched cell processes by which the cells remain in contact with neighboring osteocytes, capillaries, and osteoblasts lining the bone surface. Osteogenic cells have a highly developed cytoskeleton, and it is known that their differentiation is regulated in part through mechanical forces imposed by their surrounding environment

Methods: Hybrid silica/chitosan (CS) scaffolds were prepared by sol gel method. Conversion of hybrids to their respective aerogels was accomplished by supercritical CO2 drying. SiO2/CS composites were nominated SCS8_A, 8 being the weight percentage of CS with respect to SiO2; Physical, textural and thermogravimetric analysis, FTIR spectroscopy and swelling kinetics assays were performed. Mechanical properties were characterised by uniaxial compression, and bioactivity assays were performed in SBF. HOB® cells were seeded on the preselected scaffolds under sterile conditions, analyzed for cell viability and immunolabelled with rhodamine-phalloidin in order to assess cytoskeletal and morphological changes examined under confocal microscope

Results: Morphological analysis of shape parameters revealed changes in cell area, mainly in the cells grown in the presence of SCS8A, which are the biggest at initial times and elongate with time, as confirmed by perimeter and aspect ratio data. Osteoblasts grown in control groups and in biomaterials composed only by SiO2 presented the less complex cell morphology with highest circularity values.

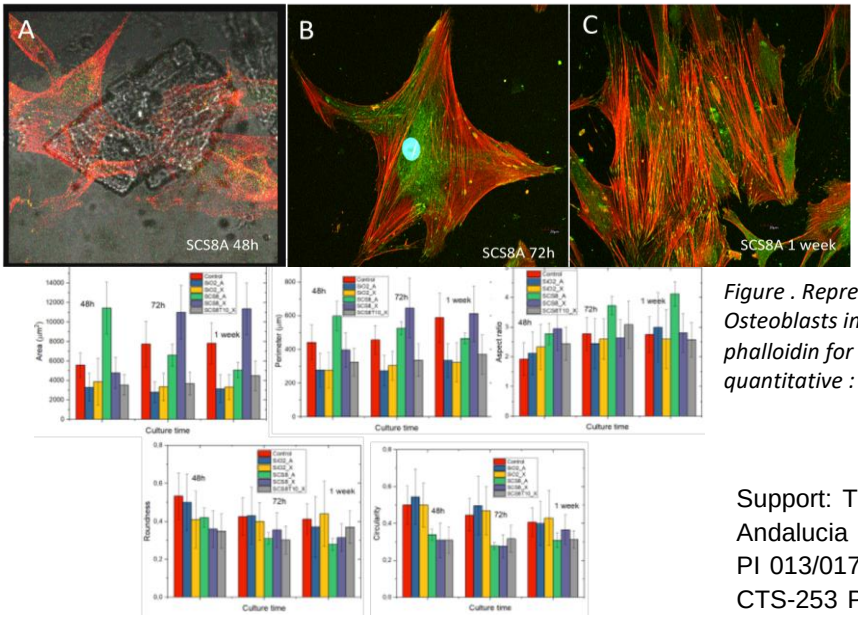


Figure . Representative images of human Osteoblasts immunolabelled with rhodamine phalloidin for actin scale bar 20 μ. and quantitative : data for shape variables

Support: This work is 80% cofinanciated by Andalucia FEDER ITI 2014-2020 Grant for PI 013/017 and Junta de Andalucia(Spain) : CTS-253 PAIDI (Tissue Engineering Group) and TEP115 Group

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Focal adhesion development and maturation in osteoblasts grown on Silica chitosan hybrid scaffolds (aerogels and xerogels) designed for bone tissue engineering

Pérez-Moreno A.[4], Reyes-Peces M.V.[4,7], Montesinos R.[1,2], Mesa-Díaz M.M.[6], de la Orden E.[1,2,3], García Palomeque J.[1,2], García Gomez N.[1], Vilches-Pérez J.I.[1,2,3], de la Rosa-Fox N.[4,5,7], Piñero-de los Ríos M.[4,5,7] and Salido-Peracaula M.[1,2,3]

[1]Department of Histology, School of Medicine, SCIB, University of Cádiz, Spain. [2] CTS253 Group, Andalusian Research Program (PAIDI). [3] Cadiz Biomedical Research Institute (INIBICA) Group EM26, Tissue Engineering. [4]Dept. of Condensed Matter Physical University of Cádiz, Spain. [5] TEP115 Group PAIDI.[6]Dept. of Chemical Engineering and Food Technologies, University of Cádiz, Spain. [7] Electron Microscopy and Materials Institute UCA (IMEYMAT).

Introduction: The importance and development of biochemical and biophysical features regulating cell-biomaterial interaction in the early steps are of capital importance in orchestrating the complex material-cytoskeleton crosstalk occurring at the interface. Focal adhesions (Fas) play a role in the process of surface recognition, with subsequent cell adhesion and mechanotransduction-induced changes in osteoblastic cells.

Methods: Hybrid silica/chitosan (CS) scaffolds were prepared by sol gel method. Conversion of hybrids to their respective aerogels was accomplished by supercritical (SC) CO2 drying. SiO2/CS composites were nominated SCS8_A, 8 being the weight percentage of CS with respect to SiO2; For xerogels synthesis, Silica/CS/TCP and Silica/CS sols were prepared using the appropriate amount of CS and TCP to obtain sols with 8% CS and 10% TCP, and dried in oven at 50 C for 28 days, to obtain SCS8_X and SCS8T10_X xerogels. Mechanical and physical properties were characterised. Bioactivity assays were performed in SBF.HOB® cells were seeded on the preselected scaffolds under sterile conditions,analyzed for cell viability, immunolabelled to assess cytoskeletal changes and FAs development and examined under confocal microscope.

Results: Live dead assays revealed no cytotoxicity. After 48 h in culture, osteoblasts grown both in aerogels and xerogels groups showed focal adhesion development, mainly small and medium sized, as expression of cell migration. In aerogels groups, and also in SiO2 X groups, small focal adhesions predominate and increased after 72 h and 1 week, while medium and big sized FAs decreased with time. In the SCS8T10 X group the percentage of mature focal adhesions, remains stable for 48 h onwards and significantly increased after 1 week. Osteoblasts grown in the presence of SCS8A seem to keep migration capability, according to FAs patterns with elongated morphology along time. In control cells, small and medium sized FAs predominate at any experimental times.

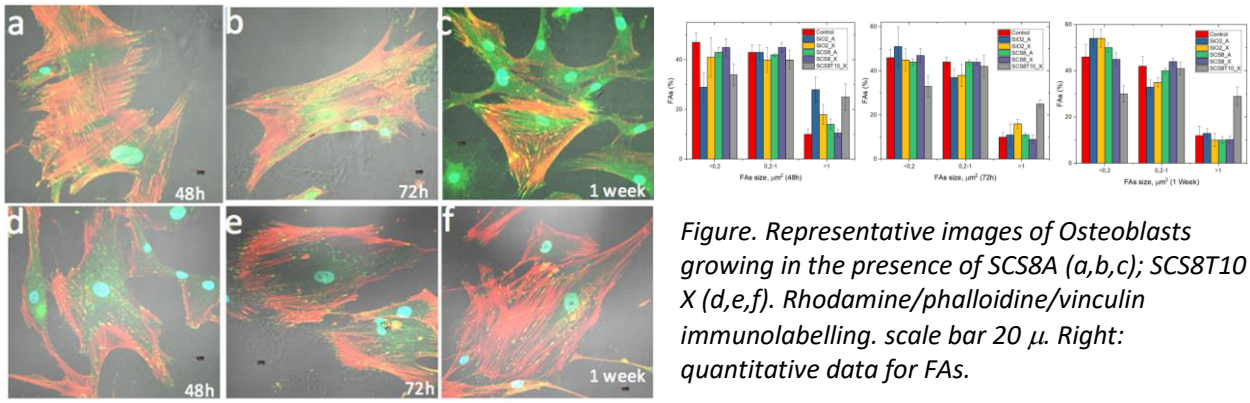


Figure. Representative images of Osteoblasts growing in the presence of SCS8A (a,b,c); SCS8T10 X (d,e,f). Rhodamine/phalloidine/vinculin immunolabelling. scale bar 20 μ. Right: quantitative data for FAs.

Support: This work is 80% cofinanciated by Andalucia FEDER ITI 2014-2020 Grant for PI 013/017 and Junta de Andalucia(Spain) :CTS-253 PAIDI (Tissue Engineering Group) and TEP115 Group.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Biological Characterization of Collagen-Silica hybrid aerogels for Bone Tissue Engineering

Mesa M.M.[6], Amaya B.[6], Reyes-Peces M.[4,7], Fernández-Montesinos R.[1,2], Garcia-Palomeque J. [1,2], García Gomez N.[1], de la Orden E.[1,2,3], Rosa Fox N.[4,5,7], Piñero de los Ríos M.[4,5,7], Vilches-Pérez J.I.[1,2,3] and Salido M.[1,2,3]

[1]Department of Histology, School of Medicine, SCIB, University of Cádiz, Spain. [2] CTS253 Group, Andalusian Research Program (PAIDI). [3] Cadiz Biomedical Research Institute (INIBICA) Group EM26, Tissue Engineering. [4]Dept. of Condensed Matter Physical University of Cádiz, Spain. [5] TEP115 Group PAIDI.[6]Dept. of Chemical Engineering and Food Technologies, University of Cádiz, Spain. [7] Electron Microscopy and Materials Institute UCA (IMEYMAT).

Bone is a hybrid material consisting of an inorganic and an organic part. The inorganic components are mainly calcium and phosphate, which form a mineral called hydroxyapatite (HA), and the organic matrix is mainly collagen. The current study addresses a composite of silica aerogel with collagen as a potential bone scaffold material for bone tissue engineering.
Methods: In order to obtain composites, we have combined collagen with TEOS, GPTMS and cellulose to synthesize, using sol-gel methods and supercritical CO_2–drying, different highly porous hybrid collagen-silica aerogels. For in vitro assays, Human osteoblasts (HOB®) cells were seeded on the preselected scaffolds under sterile conditions and after 1 week, analyzed for cell viability, immunolabelled to assess cytoskeletal changes and focal adhesion (FA) development and examined under confocal microscope.

Table 1. Properties of hybrid collagen-silica aerogels

Materials	Young´s Modulus (MPa)	BET (m^2/g)	Poro (nm)
CellSi38	6±1	644±100	22.3±2.30
CollCellSi[50/38]	41±2.0	406±19.1	32.6±1.03
CollGPSi[50/75]	90±7.1	469±14.2	10.7±1.01

Nomenclature
Cell: cellulose; Coll: collagen; GP: GPTMS; Si: silica; [% with respect to Si]

Results: Table 1 summarizes the mechanical and textural properties of aerogels. Live dead assays revealed no cytotoxicity in the presence of biomaterials. After 1 week in culture cell polarization to biomaterials until complete coverage of samples was evident with elongation, cytoskeletal changes and focal adhesion development. In CollCellSi groups morphological changes related to osteoblastc differentiation appeared to be more evident with lamellipodial and filopodial emissions and development of stress fiber tipped by numerous focal adhesions

Support: This work is 80% cofinanciated by Andalucia FEDER ITI 2014-2020 Grant for PI 013/017 and Junta de Andalucia(Spain) : CTS-253 PAIDI (Tissue Engineering Group) and TEP115 Group.

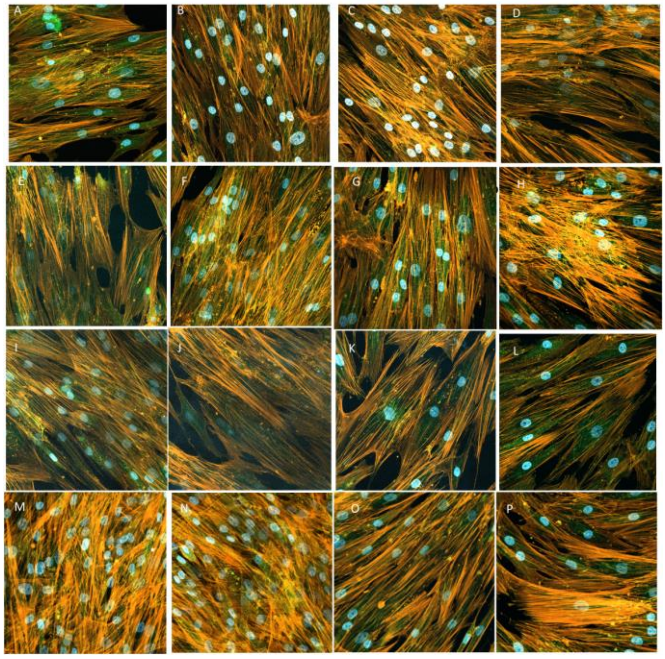


Figure. Representative images of Osteoblasts after 1 week growing in the presence of Cell Si38 samples (A,B,C,D) ; CollCellSi samples (E,F,G,H), CollGPSi samples (I,J,K,L) . Control (M,N,O,P). Rhodamine/phalloidine/vinculin immunolabelling. scale bar 20 μ.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Osteoblastic response to gelatin/GMPTS crosslinked aerogels tailored for bone tissue engineering

Reyes-Peces M.[4,7], Fernández-Montesinos R.[1,2], Mesa M.M.[6], de la Orden E.[1,2,3], Garcia-Palomeque J.[1,2], García Gomez N.[1], Rosa Fox N.[4,5,7], Piñero de los Ríos M.[4,5,7], Vilches-Pérez J.I.[1,2,3] and Salido M.[1,2,3]

[1]Department of Histology, School of Medicine, SCIB, University of Cádiz, Spain. [2] CTS253 Group, Andalusian Research Program (PAIDI). [3] Cadiz Biomedical Research Institute (INIBICA) Group EM26, Tissue Engineering. [4]Dept. of Condensed Matter Physical University of Cádiz, Spain. [5] TEP115 Group PAIDI.[6]Dept. of Chemical Engineering and Food Technologies, University of Cádiz, Spain. [7] Electron Microscopy and Materials Institute UCA (IMEYMAT).

Introduction: Bone is a hybrid material consisting of an inorganic and an organic part. The inorganic components are mainly calcium and phosphate, which form a mineral called hydroxyapatite (HA), and the organic matrix is mainly collagen. the field of bone tissue engineering (BTE) is increasingly developing new materials compatible with bone that allow bone tissue regeneration. These biomaterials must meet some requirements, such as being bioactive, biocompatible, biodegradable, and having suitable mechanical properties. We propose the synthesis of silica-gelatin hybrid aerogels (materials with high porosity and large surface area) using GPTMS as crosslinking agent.

Methods: Silica gelatin hybrid aerogels were prepared by the sol-gel method, The concentration was 20, 40 and 60 wt% gelatin to silica: G20,G40,G60. The gelatin: GPTMS ratio has been kept constant at 750:1, since it is the one that provides the best results at cellular level. Physical, textural (physisorption experiments) and termal (Thermogravimetric analysis) characterization, also FTIR spectroscopy and swelling kinetics assays were performed. Mechanical properties were characterised by uniaxial compression, and bioactivity assays were performed in SBF To investigate whether hydroxyapatite was growing on the surface. HOB® cells were seeded on the preselected scaffolds under sterile conditions., and After being incubated for 7 days, osteoblasts were analyzed for cell viability (Live/dead cell assay, Figure G to I) and immunolabeled with rhodamine-phalloidin and vinculin (Figure A to F) in order to assess cytoskeletal changes and focal adhesion development and examined under confocal microscope using Nomarski and fluorescence modes . Finally, mineralization capability was analyzed after 28 days under culture by Alizarin Red Staining Solution. Calcium deposits within cells and extracellular matrix were visualized under fluorescence microscope.

Results: Osteoblastic response in the presence of the aerogels revealed no cytotoxicity after live dead assays, with cell polarization from the first 24/48h. Actin cytoskeletal organization leads to stress fibers bundles organization and successful focal adhesion maduration. Regarding morphometry studies, osteoblasts on G60 presented the biggest area and perimeter ($p<0.05$). In addition, osteoblast in G60 were less round than those on control and G20 groups($p<0.05$). Mineralization assays on the three aerogels showed significant differences ($p<0.05$) with the negative control, which were osteoblasts grown on a glass. SEM and EDS analysis after 21 days the different samples, G20, G40 and G60, appeared to be covered by a HA layer.

Supported by CTS-253 PAIDI (Tissue Engineering Group) and Feder ITI PI013 017.

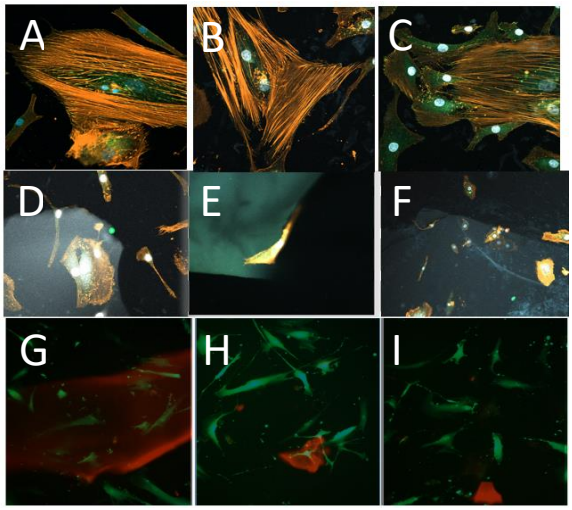


Fig. 1.Osteoblasts after 1 week growing in the presence of G20 (a,d);G40 (b,e) G60 (c,f). rhodamine phalloidin(red),vinculin (green). Blue,DAPI. In G,H,I, live dead assay scale bar 20 μ

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Bioactivity and osteoblastic response of collagen-cellulose-silica aerogels in biomedical applications

Mesa M.M.[1], Amaya B.[2], Reyes-Peces M.[2], Fernández-Montesinos R.[3], De Los Santos D.M.[5], Rosa Fox N.[2], Piñero de los Ríos M.[2], Vilches-Pérez J.I.[3,4] and Salido M.[3,4]

[1] Dept. of Chemical Engineering and Food Technologies, University of Cádiz, Spain; [2]Dept. of Condensed Matter University of Cádiz, Spain;[3]Department of Histology, University of Cádiz, Spain; [4]INIBICA Instituto de Investigación Biomédica de Cádiz, Spain; [5]Department of Physical Chemistry, University of Cádiz, Spain.

Collagen is, along with hydroxyapatite, one of the two main components of bone. As such, it has significant potential for culturing cells to produce bone. In our laboratory, we have combined collagen with silica and cellulose and using **sol-gel methods** and **supercritical CO_2 drying**, synthetized highly porous hybrid collagen-cellulose-silica aerogels. This research aims to evaluate the bioactivity and biocompatibility of aerogels in order to use them as scaffolds for bone tissue engineering. **Collagen-cellulose-silica hybrid** composites were **synthesized** in three steps: 1) collagen and cellulose was dispersed in an APTES solution (pH=4) by means of ultrasonication; 2) a silica sol was prepared by an ultrasonic-assisted sol-gel method using TEOS as a precursor; 3) both mixtures were magnetically stirred and then poured into molds, where they were aged at 50 ºC for 10 days before being dried. Different amounts of collagen and cellulose were employed (between 5-60 w/w % with respect to silica). $Ca(NO_3)_2{\cdot}4H_2O$ and KH_2PO4 were added to induce the production of hydroxyapatite. In order to determine **the tensile properties** of the Coll/Cell/Si aerogels, ten samples were tested according to ISO-527-3 specifications. The most elastic samples were those with a collagen/cellulose concentration of 50/38 %: they withstood a maximum stress of around 3652±1000 N and a strain of 90%, without breaking. The resulting Young´s modulus from strain-stress curve was 40 MPa ±1 Mpa. As it is essential that aerogels show a porous structure so that a microenvironment that allows cell adhesion and proliferation is created, their **textural properties** were figured out by the **standard BET method**. Regardless of collagen/cellulose concentration, the calculated surface areas (406±19.1 m^2/g) and the average pore diameter (32.6±1.03 nm) fluctuated within the characteristic values of mesoporous materials. The **FTIR spectra** present signal peaks associated to the functional groups: Si-O-Si (1050 cm-1), C-O (1089 cm^{-1}), Si-O-H (800 cm^{-1}), Si-O (459 cm^{-1}), N-H (1563 cm^{-1}) and C-N (1300 cm^{-1}).

The **bioactivity** of scaffolds was evaluated by examining the formation of a biologically active carbonate apatite layer on its surface after immersion in simulated body fluid (SBF) (Fig 1). **EDX spectra** results for Ca/P ratio suggested a good bioactivity of the scaffolds.

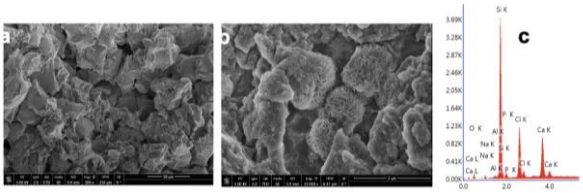


Fig. 1. a) SEM aerogels before immersion in SBF; b) after 3 weeks immersion; c) EDX spectrum.

HOB® cells were seeded on the preselected scaffolds under sterile conditions and incubated for 7 days. **Osteoblastic response** in the presence of the aerogels revealed no cytotoxicity after live dead assays and SEM imaging of osteoblasts (Fig 2) revealed cell growth, with filopodial and lamellipodial emissions polarized to aerogels. **In conclusion**, our results suggest that the prepared Cell/Si aerogels are bioactive and may be suitable for cell adhesion/attachment and, therefore, they could be used as tissue-engineering scaffolds. Cell adhesion tests are ongoing in our labs to reinforce this hypothesis

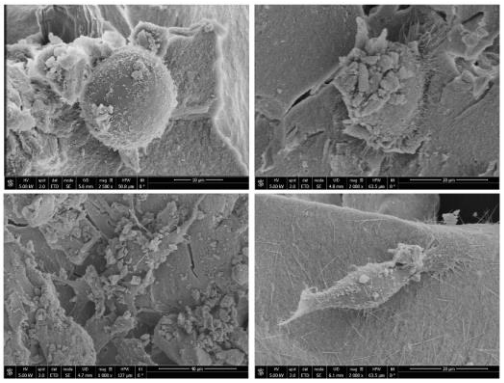


Fig. 2.SEM of osteoblasts aerogels after 1 week in culture.

Supported by CTS-253 PAIDI (Tissue Engineering Group) and Feder ITI PI013 017.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

In vitro assays of osteoblastic response to a novel biomaterial for bone tissue engineering composed by Ag nanoparticles embedded in Silica-GPTMS-Gelatin mesoporous aerogels

Reyes-Peces M.V.[4,7], Montesinos R.[1,2], Rodríguez-Núñez P.[4], Mesa-Díaz M.M.[6], de la Orden E.[1,2,3], López A.[1,2,3], García Palomeque J.[1,2], Vilches-Pérez J.I.[1,2,3], de la Rosa-Fox N.[4,5,7], Litrán R.[4], Piñero-de los Ríos M.[4,5,7] and Salido-Peracaula M.[1,2,3]

[1] Department of Histology, School of Medicine, SCIB, University of Cádiz, Spain. [2] CTS253 Group, Andalusian Research Program (PAIDI). [3] Cadiz Biomedical Research Institute (INIBICA) Group EM26, Tissue Engineering. [4] Dept. of Condensed Matter Physical University of Cádiz, Spain. [5] TEP115 Group PAIDI. [6] Dept. of Chemical Engineering and Food Technologies, University of Cádiz, Spain. [7] Electron Microscopy and Materials Institute UCA (IMEYMAT).

Introduction: Silver nanoparticles antibacterial properties when embedded in sol-gel biomaterials are intresting for bone tissue engineering. Here we report the synthesis of silica-gelatin mesoporous aerogel matrices crosslinked with 3-Glycidoxypropyltrimethoxysilane (GPTMS) to host colloidal dispersions of Ag nanoparticles, prepared by means of one-pot sol-gel processing.
Methods: First, citrate silver nanoparticles (Cit-Ag-NPs) have been prepared by a bottom-up method, starting from the reduction of AgNO3 by sodium citrate in aqueous solution. By this way, NPs with average size around 10 nm were obtained and added to the silica-GPTMS-gelatin sol matrix until gelation takes place. Silica hybrid aerogels with 25 % and 50 wt % gelatin content and GPTMS/Gelatin constant molar ratio of 500 were prepared, incorporating two different NPs aqueous solutions, with concentrations of 1mM (High, H) and 0.5 mM (Low, L), respectively (samples G50C500, C: G50C500L*; D:* G50C1000H). Wet Ag-doped gel samples were then dried with supercritical CO2 and successfully transformed to aerogels, for preventing pore collapse and preserving monolicity. HOB® cells were seeded on the preselected scaffolds under sterile conditions and analyzed for cell viability (Live/dead cell assay) after 72 h in culture to assess cytotoxicity
Results: Textural analysis from N2 phyisisorption denoted in all cases the existence of an interconnected mesopore structure with specific surface area and pore size modified by the NPs, ranging from 400 to 700 m2/g and 6 to 13 nm, respectively. In addition, FTIR spectra analysis suggested the formation of covalent crosslinked structures consisted of interpenetrated organic and inorganic networks. TEM observations confirmed that the size of NPs was around 10 nm. Additionally, live dead assays revealed no cytotoxicity, showing in all cases values quite near to positive controls. In the first 24-48 h osteoblasts polarized to materials and then started to colonize the Ag-doped aerogel samples, showing morphological changes compatible with osteocytic differentiation. Ongoing studies are developed to test antibacterial results.

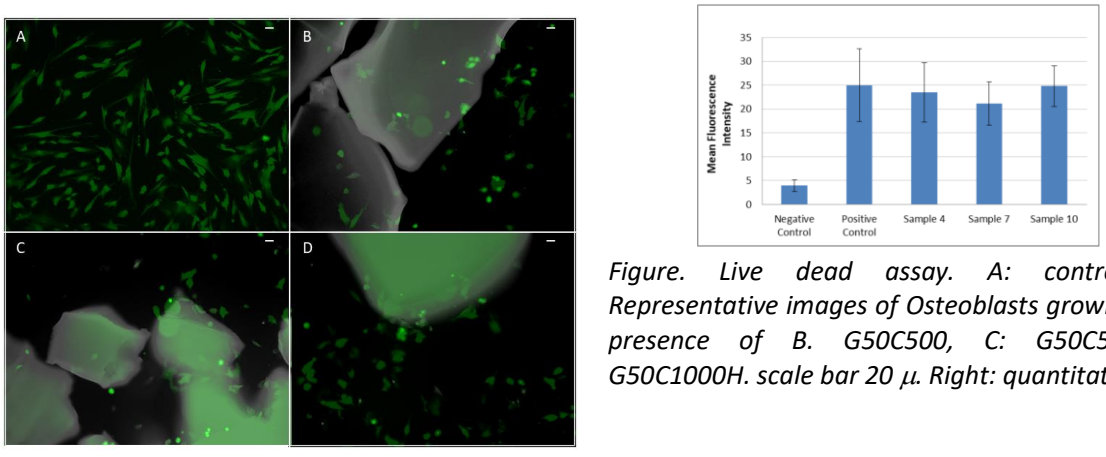


Figure. Live dead assay. A: control cells. Representative images of Osteoblasts growing in the presence of B. G50C500, C: G50C500L; D: G50C1000H. scale bar 20 μ. Right: quantitative data.

Support: This work is 80% cofinanciated by Andalucia FEDER ITI 2014-2020 Grant for PI 013/017 and Junta de Andalucia (Spain): CTS-253 PAIDI (Tissue Engineering Group) and TEP115 Group.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Biomimetic marine-based collagen/chitin nanocrystals 3D scaffolds as *in vitro* biocompatible biomaterials for bone regeneration

Olza S.[1,2,3], Fernandes S.C.M.[2,3] and Alonso-Varona A.[1]

[1] Tissue Engineering Group, Department of Cell Biology and Histology, Faculty of Medicine and Nursing, University of The Basque Country (UPV/EHU), Leioa, Spain; [2] E2S UPPA, CNRS, IPREM, Universite de Pau et des Pays de l´Adour (UPPA), Pau, France; E2S UPPA, Marine Materials Research Group, UPPA, Anglet, France

Introduction: The new frontier in the design of biomaterials for Tissue Engineering (TE) is based on the principle of biomimicry, bioinspiration and bioactivity, which aims to reproduce the *in vivo* microenvironment by mimicking the properties of the extracellular matrix (ECM) [1]. Marine organisms appeared as a new source of components with applications in bone TE. Among them, jellyfish collagen represents a potential substitute for mammalian collagen [2] and chitin nanocrystals have been reported as good *nanofillers* in biological matrices [3]. Regarding the biological component, recent studies have suggested the use of 3D culture systems of human adipose-derived mesenchymal stem cells (hASCs) for bone regeneration as these aggregates mimic more accurately the physiology in bone development [4]. The present work is focused on the analysis of the *in vitro* cytotoxicity of the marine-based scaffold, and in the evaluation of the suitability of its microporous structure as a biomimetic matrix to support the integration of previously differentiated hASCs spheroids.
Methods: The marine-based 3D macroporous scaffolds were synthesized by freeze-drying method using a mixture of jellyfish collagen (JC) and chitin nanocrystals (CHNC), crosslinked with 1%wt. EDC. The presence of the CHNC was confirmed by FTIR-ATR spectra and the macroporisity and pore size was analyzed by SEM microscopy. *In vitro* cytotoxicity tests were carried out following the rule ISO 10993 using L929 cells. In order to determine the best spheroid-forming hASC density following the hanging-drop technique, three different spheroid hASCs densities were tested by cultivation on complete culture media or osteogenic differentiation media for 21 days. The volume and degree of differentiation were evaluated at 7, 14 and 21 days.
Results: The chemical composition analysis of the scaffolds confirmed that CHNC were well integrated within the JC and the scaffolds showed a good macrostructure. SEM micrographs revealed an interconnected pore inner-structure with an average pore size higher than 150 µm. The *in vitro* cytotoxicity tests confirmed the biocompatibility of the scaffolds with and without the CHNC. The volume of the hASCs spheroids decreased throughout the time of differentiation, being the differentiated spheroids bigger than the non-differentiated ones.
Discussion & conclusions: These results suggest that JC/CHNC scaffolds present an adequate macrostructure and average pore size to seed cells or spheroids on them. Furthermore, the incorporation of CHNC doesn´t reduce the excellent intrinsic biocompatibility of the JC. The lowest density of $5 \cdot 10^3$ hASCS per spheroid showed the optimal volume to be well integrated on the scaffolds. Therefore, the JC/CHNC scaffolds are suggested as potential and adequate biomaterials for bone TE in combination with hASC spheroids. Further analysis is required to complete the characterization of the mechanical and bioactive properties of the biomaterial.

Acknowledgments: Supported by MERS and E2S/UPPA (project in cotutelle UPPA-UPV/EHU); and by UPV/EHU (PES 21/50 and GIU 19/088).

[1] Nicolas J., Magli S., Rabbachin L., Sampaolesi S., Nicotra F. and Russo L. (2020). 3D Extracellular matrix mimics: Fundamental concepts and role of materials chemistry to influence stem cell fate. Biomacromolecules 21, 1968-1994.
[2] Hoyer B., Bernhardt A., Heinemann S., Sewing J., Klinger M., Notbohm H. and Gelinsky M. (2014). Jellyfish collagen scaffolds for cartilage tissue engineering. Acta Biomater. 10, 883-892.
[3] Zubillaga V., Salaberria AM., Palomares T., Alonso-Varona A., Kootala S., Labidi J. and Fernandes SCM. (2018). Chitin nanoforms provide mechanical and topological cues to support growth of human adipose stem cells in chitosan matrices. Biomacromolecules, 19, 3000–3012.
[4] Gionet-Gonzales MA. and Leach JK. (2018). Engineering principles for guiding spheroid function in the regeneration of bone, cartilage, and skin. Biomed. Mater. 13(3): 034109.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Design of biomimetic microgels of the lung tumor microenvironment for the culture of cancer-associated fibroblasts

Monleón-Guinot I.[1,2], Milián L.[1,2], Martín de Llano J.J.[1,2], Sancho-Tello M.[1,2], García-Briega I.[3,4], Ródenas-Rochina J.[3], Gómez Ribelles J.L.[3,4], Carda C.[1,2,4] and Mata M.[1,2,5]

[1] Histopathology and Tissue Engineering Research Group (GIHIT), Department of Pathology, University of Valencia, Valencia, Spain; [2] INCLIVA Biomedical Research Institute, Valencia, Spain; [3] Centre for Biomaterials and Tissue Engineering (CBIT), Universitat Politècnica de València, Valencia, Spain; [4] Biomedical Research Networking Centre on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain; [5] Biomedical Research Networking Center of Respiratory Diseases (CIBERES), Madrid, Spain.

Many of the histopathological features of lung cancer, including metastases and drug resistance, are conditioned by the complexity of the tumor microenvironment (TME). One of the key elements conforming TME are the cancer-associated fibroblasts (CAFs). Currently, most three-dimensional (3D) culture platforms that attempt to represent cancer models still lack a complete representation of the tumor-specific extracellular matrix (ECM) [1,2]. This study attempts to generate a biomimetic culture platform to study the relationship between CAFs and other TME components, to be used as a system to study the complexity of lung cancer. Our approach consists of a microgel in which the CAFs adhere to microspheres, which represent the tumor matrix. This system would allow cell mobility, the diffusion of water-soluble molecules and the expansion of the support that is required to accommodate the formation of the new ECM produced by the cells. The microgel was composed of alginate microspheres produced in a flow-focusing microfluidic device, and whose surface was functionalized through a layer-by-layer process to allow cell adhesion (Fig.1). To validate the model, CAFs were cultured on the microgel and, after three days, the characteristics of the nucleus and cytoskeleton of the cells were studied under the fluorescence microscope. The results indicate that the cells bind to the microgel and interact with each other forming networks that surround each microsphere surface. We consider that this model shows promising results as a 3D culture platform that could be in the future, after further investigations, an *in vitro* disease model for lung cancer.

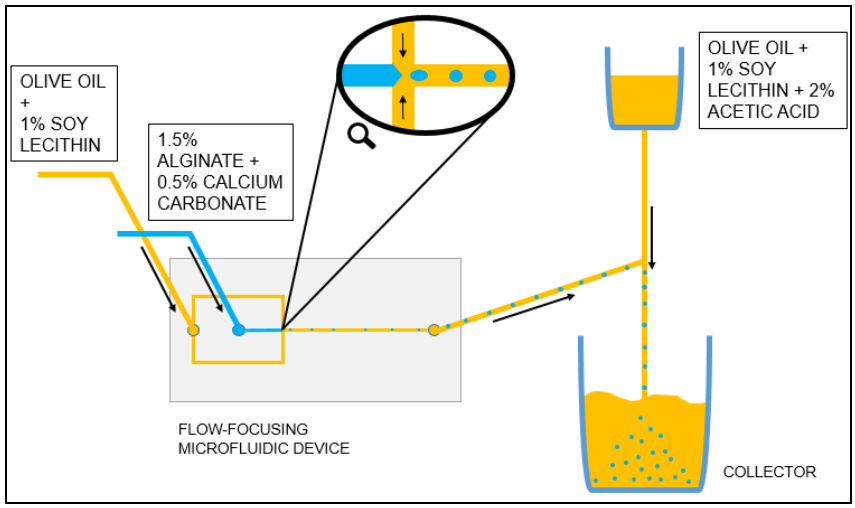


Fig. 1. Schematic representation of the flow-focusing microfluidic device.

[1] Ferreira L.P., Gaspar V.M. and Mano J.F. (2018). Design of spherically structured 3D in vitro tumor models-Advances and prospects. Acta Biomater. 75:11-34.
[2] Thoma C.R., Zimmermann M., Agarkova I., Kelm J.M. and Krek W. (2014). 3D cell culture systems modeling tumor growth determinants in cancer target discovery. Adv Drug Deliv Rev. 69-70:29-41.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Development of organoids of co-cultures of A549 and Cancer Associated Fibroblast (CAFs) to study lung cancer microenvironment

Martín de Llano J.J.[1,2], Martínez-Vallejo P.[1], Milián L.[1,2], Monleón I.[1,2], Oguir Z.[1,2], Sancho-Tello M.[1,2], Carda C.[1,2,3] and Mata M.[1,2,4]

[1] Department of Pathology. Faculty of Medicine and Dentistry. Universitat de València. Valencia, Spain; [2] INCLIVA Biomedical Research Institute. Valencia, Spain; [3] CIBER-BBN. Valencia, Spain; [4] CIBERES. Madrid, Spain

Introduction: Lung cancer is the leading cause of cancer death worldwide. There are multiple factors that determine the ability of tumor cells to disseminate and nest in different organs, thus generating metastases. One of these essential factors is the tumor microenvironment (TME). Cancer Associated Fibroblasts (CAFs) are involved in the control and modification of the tumor stroma, affecting a variety of tumor cells characteristics, such as the epithelial-to-mesenchymal transition (EMT) potential and cell survival. The aim of our study is to develop 3D co-cultures of lung carcinoma cells and CAFs isolated from lung cancer patients that can be used to expand our knowledge of the EMT. In the next future, the objective is to create patient specific organoids that allow us to develop personalized studies, as specific anticancer drugs.

Methods: CAFs were obtained from patients diagnosed with lung adenocarcinoma. A549 is a lung carcinoma cell line. Cells were cultured and expanded following standard procedures. Cell spheroids were obtained according to the hanging-drop procedure of Conti et al. [1]. Briefly, cells were resuspended and mixed to obtain a suspension of 1.5×10^5 cells/mL. Drops of this suspension were pipetted on the internal surface of a Petri dish lid. The lid was placed on the dish (drops upside-down) and after 72 h of cell culture the spheroids were collected and dispersed in a collagen type I solution. One drop of the spheroids suspension was pipetted on each well of a 12-well cell culture plate. The plate was incubated for 2 min and then inverted and incubated for 2 min more. These steps were repeated until the collagen polymerized, thus originating the organoids, spheroids embedded in a matrix. Cell culture medium was added to the organoids, that were cultured following standard procedures and monitored by phase-contrast microscopy. At selected times the organoids were processed to visualized F-actin, vimentin and keratin expression by fluorescence microscopy.

Results: Organoids containing A549 cells, CAFs and mixtures of both cell types (Fig. 1) were consistently obtained. Spheroid size increased with cell culture time. Cells on the surface of the spheroids containing CAFs started to show a cell-migrating morphology after 24 h of cell culture and matrix invasion increased at longer cell culture times. A549 cells expressed keratin and F-actin, and heterogeneously vimentin. CAFs expressed F-actin and vimentin. A549 and CAFs cells were distinguished in mixed spheroids through the analysis of the expression of F-actin and keratins by immunofluorescence.

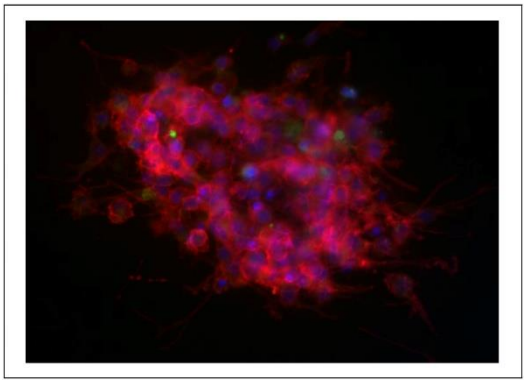


Fig. 1. An organoid containing A549 and CAFs cells. F-actin and pankeratin immunofluorescence (red and green color, respectively) and DAPI stained nuclei.

Discussion and conclusions: A549 morphological and phenotype changes in the presence of CAFs cells can be followed by analyzing mixed spheroids embedded in a collagen matrix as described herein. The use of CAFs obtained from a lung cancer patient will allow to study how these specific CAFs affect the EMT of A549 cells and, eventually, help to develop specific treatments for that lung cancer.

Supported by grant PID2019-106099RB-C42 (MM) from the Ministry of Economy and Competitiveness of the Spanish Government.

[1] Conti S., et al. The Epithelial- to Mesenchymal Transition. Methods in Molecular Biology (Clifton, N.J.) v.2179, chapter 19.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Generation and characterization of 3D microtissues of L929 murine fibroblast cells for tissue engineering and pharmacological applications

Andrade F.R.S.[1,2], Lucena E.S.[1,2], Marinho A.D.[1,2], Oliveira A.C.X.[1,2], Monteiro H.S.A.[1,2], Sánchez-Porras D.[3,4], García-García O.D.[3,4], Bermejo-Casares F.[3], Montenegro R.C.[1,2], Carriel V.[3,4] and Jorge R.J.B.[1,2]

[1] Pharmacology Group, Department of Physiology and Pharmacology, Federal University of Ceará, Ceará, Brazil; [2] Drug Research and Development Center, Federal University of Ceará - Fortaleza, Ceará, Brazil; [3] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [4] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain

Introduction: Cells models are important for drug and disease studies according to the 3R rules (Refinement, Reduction, Replacement) [1]. *In vitro* three-dimensional (3D) cell culture systems can better mimic *in vivo* physiology and tissue microenvironment more than two-dimensional or monolayer (2D) [2]. The objective of this study is to generate and characterize 3D microtissues of L929 murine fibroblast cells for tissue engineering and prediction of drug applications.

Methods: L929 microtissues were generated in three concentrations ($1.25x10^4$, $2.5x10^4$ and $5x10^4$ cells per chip) using agarose chips and evaluated during a 15 days period of time *in vitro* through qualitative and morphometric analysis (solidity, circularity and Feret's diameter). Cell viability tests (differential acridine orange/ethidium bromide (AO/EB) staining) and histological analysis (hematoxylin-eosin, alcian blue, picrosirius and Ki-67) were assessed in the concentration that showed the best growth characteristics ($2.5x10^4$ cells per chip).

Results: Qualitative observation suggested that the fifth day of culture marked the initial formation of the microtissue, presenting more compacted cells. On the tenth day, the microtissue was more stable and with more regular edges. The fifteenth day, however, cells showed excessive growth reaching the limits of the edges of the microwells. Morphometric evaluation indicated a variation from 0.92 to 1.00 in solidity, 0.72 to 1.00 in circularity and 570.62 to 1,284.06 in Feret's diameter at 5^{th} and 15^{th} days, respectively. Fluorescence microscopy analysis revealed that the microtissues kept their viability until the tenth day of culture (Fig. 1). Histochemical results demonstrated that microtissue-forming L929 cells did not deposit collagen fibers but a low amount of proteoglycans was observed. Ki-67 immunohistochemistry revealed an active cell proliferation in all days analyzed.

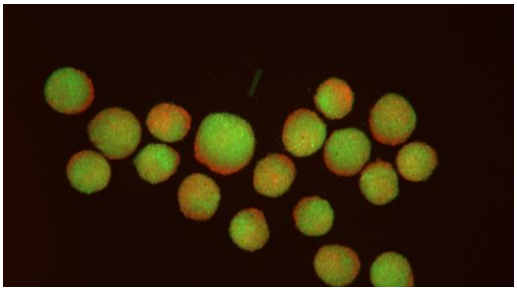


Fig. 1. Tenth day microtissues viability test - differential acridine orange/ethidium bromide (AO/EB) staining.

Discussion: Previously published results [3] proved that the primary culture of fibroblasts remained viable until the 28th day of growth, presented a smaller size when compared to the L929 strain and expressed abundant collagen production, indicating the presence of extracellular matrix in the microtissues. This study is ongoing to assess whether these cells are capable of generating collagen fibers and proteoglycans, important components of the extracellular matrix. The present 3D model can be used to mimic healthy or diseased tissues, contributing as a novel tool for toxicological screening, wound healing and drug prediction studies.

Fundings: This study was supported by grant PI-0257-2017 from Consejería de Salud y Familias, Junta de Andalucía, Spain and CTS-115 (Tissue Engineering Group). Bezerra-Jorge R.J. was supported by Coimbra Scholarship Programme for Young Professors and Researchers from Latin American Universities.

[1] Saglam-Metiner, P., et. al. (2019). Gene, 686, 203-212
[2] Jensen, C., & Teng, Y. (2020). Front. Mol. Biosci. 7:33. doi: 10.3389/fmolb.2020.00033
[3] Durand-Herrera, D. et al. (2018). Histochem Cell Biol., 150(4), 379-393

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Organ on chip (OoC): recreation of complex histological architecture in preclinical *in vitro* models

Oliván S., Abizanda S., Bayona C., Castro H., González S., Lacueva A., Olaizola C., Paz L., Randelovic T., Sharko V. and Ochoa I.

TMELab, I3A_IIS Aragón. CIBER-BBN. Department of Human Anatomy and Histology. University of Zaragoza. Zaragoza, Spain.

INTRODUCTION: The different tissue structures of our body combine multiple cell types with spatially defined cell distributions. This complex configuration is hardly mimicked in vitro with our classical in vitro models based on 2D cultures in Petri dish plasticware. However, in the last decades, a new multidisciplinary technology has emerged to mimic human physiology in vitro at the cell scale (microns). This technology, called organ on a chip (OoC) or Microphysiological Systems (MPS), integrates microfluidic devices with complex cell culture techniques (hydrogels, organoids, or even co-cultures) and mechano-physiological conditions to recreate the tissue microenvironment[1]. When using OoC technology, multiple advantages can also be found, such as minimising the materials and reagents needed, allowing cellular interaction with the extracellular matrix, better controlling critical parameters through integrated sensors, or incorporating electrical and mechanical stimuli[2]. The establishment of the most suitable in vitro model provides a reduction in times, costs and, last but not least, in the number of animal experiments as recommended by the 3Rs (replace, reduce, refine) ethical guiding principles for testing involving animals. The present work presents an overview of different experimental models developed in our laboratory based on microfluidic devices simulating several histological microenvironments in patho/physiological conditions.

METHODS: In recent years, the development of microfluidic technology is allowing a more precise simulation of the complex cellular environment. This technology is capable of recreating physiological environments on the microscale with microfluidic devices made of plastic or elastomeric materials that replace the classic 2D cell culture plate. Microdevices are capable of generating channels and chambers on the scale of the cell and, therefore, allow cultures to be organized in a 3D manner. In addition, these devices allow different external stimuli to be applied, simulating the physiological conditions (oxygen gradient, mechanical deformation, shear stress in the vascular area...) of the organ under study. Our group has developed several models related to the colon, skin or renal system and simulated pathological diseases such as glioblastoma (figure 1).

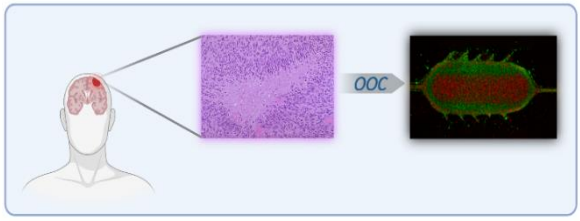


Figure 1. Glioblastoma-on-a-Chip Model

CONCLUSIONS: Thanks to the design versatility and simple manufacturing of these new "organ-on-chip" devices, it has been possible to simulate multiple cellular behaviours as relevant as cell migration, response to drugs in different metabolic environments, antitumor efficacy in complex models with tumour-vascular interaction, the processes of extravasation of cells of the immune system or the progression of a tumour in vitro[1-2]. Furthermore, these devices have the advantage that they have very small culture chambers and, therefore, the number of cells required to carry out the studies is also very low. This fact therefore allows the use of cells obtained directly from the patient (biopsies and/or blood samples), which is a further step towards personalized medicine.

*Acknowledge: BEOnChip S.L. and Servicio General de Apoyo a la Investigación-SAI, Universidad de Zaragoza. Supported by PRIME (H2020-FETOPEN-2018-2019-2020-01) RISE CISTEM (H2020-MSCA-RISE-2017 No.778354) and M4M (**H2020-EU.2.1.1.7. – ECSEL** ID: 876190) projects.*

[1] Ingber, D. E. Nat. Rev. Genet. 0123456789, (2022).
[2] Zhang, B. Nat. Rev. Mater. 3, 257–278 (2018).

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Influence of serum presence on the neurodifferentiation process of human dental pulp stem cells

Pardo-Rodriguez B.[1], Manero-Roig I.[1,2], Luzuriaga J.[1], Polo Y.[3], Romayor I.[1], Unda F.[1], Ibarretxe G.[*1] and Pineda J.R.[*1,4]

[1]University of the Basque Country (UPV/EHU), Leioa, Spain; [2]Université de Bordeaux IINS - UMR 5297, Bordeaux, France; [3]Polimerbio SL, Donostia-San Sebastian, Spain; [4]Achucarro Basque Center for Neuroscience Fundazioa, Leioa, Spain. * Corresponding authors

Introduction: Human dental pulp stem cells (hDPSCs) constitute one of the most promising alternatives for neuroregenerative therapies. Unlike other human stem cells, hDPSCs can be very efficiently differentiated without a need of genetical engineering to neural cells secreting anti-inflammatory and neuroprotective factors and expressing mature neuronal or glial markers [1]. Moreover, neurodifferentiated hDPSCs possess voltage dependent channels and neurotransmitter receptors, which make them capable to respond to CNS signals and even fire action potentials [2]. However, a better understanding of their true capacity of integration into the synaptic network is still required, and hDPSC *ex vivo* expansion and differentiation protocols must be refined. The inclusion of fetal bovine serum (FBS) in cell culture media is a widespread practice that helps to improve hDPSC proliferation and survival. However, this also comes at the expense of a loss of neural differentiation capacity [3]. Thus, in this study we assessed the reversibility of FBS-induced changes when hDPSCs were switched from serum-containing to serum-free media.
Methods: hDPSCs were extracted from the third molar of healthy donors. Cells from the same patient were cultured for three weeks in proliferation media either with or without 10% FBS. Then, sister cultures were switched to serum-free Neurocult differentiation™ medium and cells were fixed or frozen at three different time points: DIV7, DIV14 and DIV21. At each time point in both conditions different stem cell, glial, neuronal and synaptic markers were compared: human-Nestin, glial fibrillary acidic protein (GFAP), S100β, p75, Olig2, doublecortin (DCX), NeuN and Synapsin-I.
Results: We observed that after switching hDPSC cultures to serum-free media and inducing their neurodifferentiation, cells that had been previously grown in serum-containing and serum-free media were morphologically very different, and showed distinctive marker expression patterns. Cells cultured in a serum containing media showed an increased nuclear size and fibroblast-like cytoplasmic morphologies. By contrast, hDPSCs previously cultured without serum showed smaller cell bodies with many long, thin and ramifying processes. Besides, cells that had been previously expanded in a serum-free medium showed staining for DCX, NeuN and synapsin-I neuronal markers after 21 days of differentiation. The expression of synaptic markers was not detected in hDPSC cultures that had been previously expanded in the presence of FBS.
Discusion and conclusions: Our results highlight the importance of choosing an appropriate differentiation protocol that determines the neural fate of hDPSCs, paying particular attention to the presence of FBS. The *in vitro* expression of synaptic proteins suggests the possibility that hDPSC-derived cells may be capable of integrating into the brain synaptic network, which could be a decisive factor in the potential use of hDPSCs for neuroregenerative cell therapies.

Supported by UPV/EHU (PPGA20/22) Basque Government Health Department (Grant 2021333012), MICINN/AEI/10.13039/50110001103 (PID2019-104766RB-C21 & MINECO RYC-2013-13450). B. Pardo-Rodriguez has a predoctoral funding from the Basque Government (Ref. 2021_1_0112). Polimerbio and Y. P. have a Bikaintek PhD grant (20-AF-W2-2018-00001). I.M. have a UPV/EHU mod-III grant. I.R have a Margarita Salas Grant (MARSA21/60).

[1] Luzuriaga J, et al. Int J Mol Sci. 2021,22(7):3546.
[2] Gervois P, et al Stem Cells. 2015, 24(3):296-311.
[3] Pisciotta A, et al. PLoS One. 2012, 7(11):e50542.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Histological characterization of mesenchymal stem cells of the umbilical cord for use in tissue engineering

Blanco-Elices C.[1,2,3], Ortiz-Arrabal O.[2,3], García-García O.D.[2,3], González-Gallardo C.[3,4], Oyonarte S.[2,3,5], de la Cueva-Batanero P.[2,3], Alaminos M.[2,3] Martín-Piedra M.A.[2,3], Carriel V.[2,3] and Garzón I.[2,3]

[1] Doctoral program in Biomedicine, University of Granada, Spain; [2] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [3] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [4] Division of Ophthalmology, University Hospital Virgen de las Nieves, Granada, Spain; [5] Network for Transfusional Medicine, Cells and Tissues, Granada, Spain

Introduction: The human umbilical cord is a perinatal tissue containing an abundant population of mesenchymal stem cells (MSC) with high plasticity and potential usefulness in Tissue Engineering [1]. Four topographical zones can be distinguished in the human umbilical cord: intervascular -IV-, perivascular -PV-, subamnioblastic -SAM-, and Wharton jelly -WH- zones [2] (Fig. 1). The aim of the present study is to perform a histological characterization of these zones in order to determine their potentiality for use in tissue engineering of the human cornea, oral mucosa, skin and nerve using MSC isolated from the human umbilical cord.

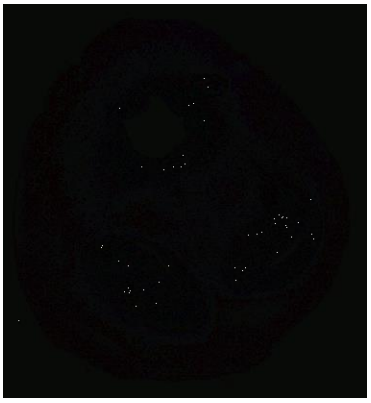


Fig. 1. Human umbilical cord section showing three blood vessels and the different topographical zones.

Methods: We carried out histological and immunohistochemical study of the human umbilical cord using several extracellular matrix markers in order to determine the potential of each specific zone in tissue engineering. In addition, MSC primary cell cultures were established from each zone of the umbilical cord and *ex vivo* analyses were performed on primary cell cultures.

Results: On the one hand, our histological results confirmed the *in situ* heterogenicity of the human umbilical cord, showing different cell density and extracellular matrix composition in the different zones of the human umbilical cord. Our results revealed that human umbilical cord cells had intrinsic capability to synthetize several components of the extracellular matrix of the umbilical cord *in situ*. However, isolation and *ex vivo* culturing resulted in a homogenous cell profile in all MSC types obtained from the different zones, although some differences were found.

Discussion & conclusions: Our results confirmed the heterogeneity of the human umbilical cord and the need of selecting cells from specific regions of the human umbilical cord for particular applications in Tissue Engineering. MSC showed different profiles *in situ*, and different biosynthetic capability for several components of the umbilical cord extracellular matrix. Once isolated, primary cultures of umbilical cord MSC corresponding to different zones resulted in a similar histological profile. These results suggest that umbilical cord MSC may display different potential according to their specific location in the cord, and that their isolation from the umbilical cord environment could modify their behavior and induce a pro-proliferative profile with few differences among zones. Therefore, MSC isolated from all zones could have similar potentiality for use in tissue engineering of the human cornea, oral mucosa, skin and nerve.

This work was supported by the Spanish Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+i) of the Spanish Ministry of Science and Innovation (Instituto de Salud Carlos III), Grants FIS PI21/0980, FIS PI18/0331, FIS PI20/0317, FIS PI20/318, and ICI19-00024, co-financed by FEDER funds (European Union). It was also supported by grant PE-0395-2019 from Consejería de Salud y Familias, Junta de Andalucía, Spain, and grants B-CTS-450-UGR20 and A-CTS-498-UGR18 (Proyectos de I+D+i en el marco del Programa Operativo FEDER Andalucía 2014-2020) from the University of Granada, Consejería de Transformación Económica, Industria, Conocimiento y Universidades, Junta de Andalucía and European Union and P18-RT-5059 from Consejería de Transformación Económica, Industria, Conocimiento y Universidades, Junta de Andalucía. Co-financed by FEDER funds (European Union).

[1] Garzón I, et al. Tissue Eng Part B Rev. 2020;26(4):301-312
[2] Garzón I, et al. Placenta. 2014;35:994-1000

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Ex vivo and in vivo histological assessment of a novel acellular nerve allografts for peripheral nerve repair

García-García O.D.[1,2], El Soury M.[3], Chato-Astrain J.[1,2], Sánchez-Porras D.[1,2], García J.M.[1,2], Raimondo S.[3], Gambarotta G.[3], Campos A.[1,2], Campos F.[1,2] and Carriel V.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] Department of Clinical and Biological Sciences and Neuroscience Institute Cavalieri Ottolenghi (NICO), University of Torino, Orbassano, Italy. Contact: ogarcia@ugr.es; vcarriel@ugr.es

Introduction: Organs decellularization has emerged as a promising alternative to generate 3D matrices to repair or substitute damaged organs. This method resulted especially useful in the treatment of complex organs such as peripheral nerve (PN) with promising *ex vivo* and *in vivo* results [1,2]. However, an optimal decellularization method for PN has not been developed, and improvements to minimizing the negative impact on the extracellular matrix (ECM) able to improve the efficacy obtained with the current gold standard autograft technique, are still needed. The goal of this study was to evaluate *in vitro* the efficiency of a novel chemical-enzymatic decellularization method (CE) for PN in comparison with the classical Sondell (SD) method. Then, the therapeutic efficacy of these new products was determined in a rat model by using the autograft technique (AUTO) and undamaged nerves (CTR) as control groups.
Methods: Wistar rat sciatic nerves were collected and decellularized using the novel CE protocol [Triton X-100, SDS, SDC and an enzymatic mix (DNase and RNase)] and the classical SD protocol [two cycles of Triton X-100 and SDC] [1,2]. The *in vitro* characterization was carried out by histological, ultrastructural and DNA quantification analyses. Subsequently, CE and SD decellularized peripheral nerve allografts were used to repair a 10-mm gap in the left sciatic nerves. After 15 weeks of surgery, the regeneration profile was evaluated by functional, histological and histomorphometrical tests.
Results: Hematoxylin-eosin staining showed a higher conservation of the stromal pattern in CE group as compared with SD. In addition, DAPI fluorescence DNA staining revealed remnants of DNA in SD group but not in CE being these differences significant ($p<0.05$). Moreover, ultrastructural analyses (TEM and SEM) demonstrated that CE groups presented a better conservation of the collagen pattern than SD with an efficient removal of the myelin in both groups. The *in vivo* evaluation showed a better degree of motor and sensory recovery in CE respect to SD group. These results were comparable but not superior to the AUTO group. In line with these findings, our histological analyses showed a more abundant nerve tissue regeneration as determined by the myelinization pattern (MCOLL), Schwann cells (S-100) and mature axons (neurofilament) in CE and AUTO groups as compared with SD. Finally, the histomorphometrical analysis showed that CE had significant superior results ($p< 0.05$) in density and total number of fibers to the classical SD group, being these findings comparable to AUTO group ($p>0.05$). However, the pattern obtained was not compared to CTR healthy nerves, as expected.
Discussion: This study demonstrates a more efficient decellularization and ECM preservation with the novel CE method than with SD. In vivo assessment confirmed superior functional, histological and histomorphometrical profiles as compared to the classical SD method, being these results comparable but not yet superior to the gold standard AUTO group. Finally, further investigations are needed to determine the efficacy of these new neural substitutes in the repair of critical injuries.

Supported by the Spanish Plan Nacional de Investigación Cientifica, Desarrollo e Innovación Tecnológica (I+D+i) of the Spanish Ministry of Science and Innovation, grant FIS PI20/0318, Instituto de Salud Carlos III. Supported by Plan Andaluz de Investigación, Desarrollo e Innovación (PAIDI 2020), Consejería de Transformación Económica, Industria, Conocimiento y Universidades, Junta de Andalucía, Spain, grants P18-RT-5059 and A-CTS-498-UGR18 (proyectos de I+D+i en el marco del Programa Operativo FEDER Andalucía 2014-2020, University of Granada). Co-financed by the European Regional Development Fund (FEDER - ERDF).

[1] Chato-Astrain J, et al. J Tissue Eng Regen Med. 2020;14(6):789-806
[2] Philips C, et al. Ann Biomed Eng. 2018;46(11):1921-1937

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Evaluation of novel 3D-printed scaffolds for neural tissue engineering applications

Etayo-Escanilla M.[1,2,3], García-García O.D.[1,2], Chato-Astrain J.[1,2], Sánchez-Porras D.[1,2], Campos F.[1,2], Baena J.M.[3,4], Vieira S.[5] Pegueroles-Neyra M.[6], Campillo N.[3,4] and Carriel V.[1,2]

[1] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; [3] REGEMAT 3D S.L., Granada, Spain; [4] BRECA Health Care S.L.[5] Institute of Biomedicine/iBiMED, Department of Medical Sciences, University of Aveiro, 3810-193 Aveiro, Portugal, [6] Biomaterials, Biomechanics and Tissue Engineering Group (BBT), Universitat Politècnica de Catalunya (UPC), Spain. Contact: lab1@regemat3d.com, vcarriel@ugr.es

Introduction: Neural damage, mainly due to spinal cord and peripheral nerve injuries, presents a high incidence in our population. They can lead to permanent or partial incapacitating conditions drastically reducing the life quality of patients worldwide [1]. Neural tissue possesses a limited intrinsic regenerative capability and a highly complex structure that have hindered their treatment and recovery [2]. Recently, 3D bioprinting has emerged as a promising alternative to produce scaffolds with anatomically accurate detailed geometries which could lead the generation of biomimetic nervous system substitutes. The aim of the present work was to analyze the usefulness of different biomaterials for the generation of 3D printed scaffolds for neural tissue engineering.
Methods: Four types of biomaterials [polycaprolactone (PCL), polylactic acid (PLA), a flexible and electroconductive thermoplastic polyurethane (FF), and a thermoplastic elastomer (FD)] were used to generated 3D porous scaffolds by using a REG4Life 3D bioprinter (REGEMAT 3D S.L.). Scaffolds were biomechanically characterized under tensile tests by using an Instron (Model 5943) and their electroconductive properties were determined with a Modern Digital Multimeter (UT58A, Uni-Trend Technology) and a Laboratory DC Power Supply (IPS 2303, ISO-TECH). For the *in-vitro* assays, human neuroblastoma cells (SK-N-AS cell line) were seeded into scaffolds and cell adhesion, morphology and viability were determined at 7 days of culture by Live/Dead assay and scanning electron microscopy (SEM).
Results: Tensile tests revealed Young's modulus (YM) and stress at fracture (SF) significant biomechanical differences among groups ($p < 0,05$). More elastic properties were obtained with FD as compared to other biomaterials. The SF mean values were proportional to those obtained the YM. However, FD showed a SF mean values similar to PCL and FF. Electroconductivity test only showed electroconductive properties in FF (0,01666 ± 0,0007 S/m). According to the Live/Dead and SEM assay, FF presented lower biocompatible properties, in terms of cell adherence and viability, than the rest of the scaffolds generated, where the results were comparable.
Discussion: FD-based 3D scaffolds showed greater biomechanical properties than the rest of the biomaterials used, presenting a better elastic behavior and a high resistance to fracture. In terms of cell adherence and viability, FD demonstrated comparable results PLA and PCL, which have already been used in neural tissue engineering [3]. For these reasons and despite it showed no electroconductive properties, FD could be proposed as a promising biomaterial for neural tissue engineering applications. Further research is necessary to evaluate their *in vivo* usefulness.

Supported by the Spanish Plan Nacional de Investigación Cientifica, Desarrollo e Innovación Tecnológica (I+D+i) of the Spanish Ministry of Science and Innovation, grant FIS PI20/0318, Instituto de Salud Carlos III. Supported by Plan Andaluz de Investigación, Desarrollo e Innovación (PAIDI 2020), Consejería de Transformación Económica, Industria, Conocimiento y Universidades, Junta de Andalucía, Spain, grants P18-RT-5059 and A-CTS-498-UGR18 (proyectos de I+D+i en el marco del Programa Operativo FEDER Andalucía 2014-2020, University of Granada). Grant PTQ2019-010731, Ayudas Torres Quevedo 2019, Plan Estatal de Investigación Científica y Técnica y de Innovación 2017-2020, Ministry of Science and Innovation, Spain. Co-financed by the European Regional Development Fund (FEDER - ERDF).

[1] Bedir T., et al. Mater Sci Eng C Mater Biol Appl. 2020;110:110741
[2] Chato-Astrain J., et al. Basic nerve histology and histological analyses following peripheral nerve repair and regeneration. Peripheral Nerve Tissue Engineering and Regeneration. Reference Series in Biomedical Engineering. Springer, Cham. 2022; https://doi.org/10.1007/978-3-030-21052-6_14
[3] Yu X, et al. Polymers. 2020;8:1637

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Chitosan conduits filled with fibrin/collagen hydrogels with or without Adipose Mesenchymal Stem Cells for Peripheral Nerve Repair

García-García O.D.[1,2,§], El Soury M.[3,4,§], Campos A.[1,2], Tarulli I.[3], Chato-Astrian J.[1,2], Perroteau I.[3], Geuna S.[3,4], Raimondo S.[3,4], Gambarotta G.[3,4] and Carriel V.[1,2]

[1] Tissue Engineering Group,Department of Histology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria, Ibs.GRANADA, Granada, Spain; [3] Nerve regeneration Group, Department of Clinical and Biological Sciences, University of Torino, Torino, Italy; [4] Neuroscience Institute Cavalieri Ottolenghi (NICO), University of Torino, Torino, Italy; [§] equal contribution. Contact: ogarcia@ugr.es

Introduction: Repairing severe peripheral nerve injuries remains a great challenge for surgeons, since regeneration outcomes are not usually satisfactory and complete functional recovery is rarely achieved [1]. In case of nerve injuries accompanied by substance loss, nerve autografts are used to reconnect both transected nerve stumps. Tubulization technique is an alternative that has been developed to repair nerves and to overcome the limitations accompanying the use of autografts. It is highly efficient in repairing small gaps (up to 3 cm), while to improve the conduit efficiency in repairing long gaps intraluminal enrichment might be a good strategy [2]. The objective of this study was to evaluate the potential usefulness of chitosan conduits filled with a hybrid hydrogel composed by fibrin/collagen (F/C) and adipose mesenchymal stem cells (ADMSC) in the repair of 15-mm sciatic nerve injuries in rats.
Methods: Under general anesthesia a 15-mm nerve defect was created in each animal and the defects were repaired by using i) Hollow Chitosan Conduits; ii) Conduits filled with acellular hydrogels; and iii) conduits filled with F/C hydrogels containing ADMSC. Regeneration was evaluated by functional, histological and molecular analyses at 15 weeks.
Results: The molecular assessment of various growth (NRG1 & VEGF-A) or transcription factors (c-Jun, Egr2 and ATF3) at early stages of regeneration (7, 14 and 28 days) has shown positive results in F/C + ADMSC group. However, functional assessment conducted at 15 weeks did not reveal acceptable nerve and muscle functional recovery, being these results more favorable with the use of hollow conduits. Histology confirmed these findings showing poor sings of nerve tissue regeneration crossing the gaps.
Discussion: This study revealed negative result with the combination of chitosan conduits filled with F/C hydrogels in the repair of 15-mm nerve gaps in rats. Histology confirmed that the formation of an intraluminal tissue was considerably affected, and it could be related to inadequate structural properties of the biomaterials used, fast F/C biodegradation, inadequate combination strategy or the well-known natural hydrogel contraction process. This study clearly confirm that further studies are needed to optimize the hydrogel structural, chemical and biological properties of biomaterials for use in nerve repair.

Supported by the Spanish Plan Nacional de Investigación Cientifica, Desarrollo e Innovación Tecnológica (I+D+i) of the Spanish Ministry of Science and Innovation, grant FIS PI20/0318, Instituto de Salud Carlos III. Supported by Plan Andaluz de Investigación, Desarrollo e Innovación (PAIDI 2020), Consejería de Transformación Económica, Industria, Conocimiento y Universidades, Junta de Andalucía, Spain, grants P18-RT-5059 and A-CTS-498-UGR18 (proyectos de I+D+i en el marco del Programa Operativo FEDER Andalucía 2014-2020, University of Granada). Co-financed by the European Regional Development Fund (FEDER - ERDF).

[1] Carriel V., et. al. J Tissue Eng Regen Med. 2017;11(2):553-563
[2] García-García O.D., et al. Int J Mol Sci. 2021 2021;22(2):674

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Comparison of decellularization protocols to generate peripheral nerve-derived 3D extracellular matrices for neural engineering

El Soury M.[1,2,*], García-García Ó.D.[3,4,*], Moretti M.[5,6], Perroteau I.[1], de la Cueva-Batanero P.[3], Oyonarte S.[3,4], Campos A.[3,4,7], Raimondo S.[1,2], Lovati A.B.[5] and Carriel V.[3,4]

[1] Nerve regeneration Group, Department of Clinical and Biological Sciences, University of Torino, Torino, Italy; [2] Neuroscience Institute Cavalieri Ottolenghi (NICO), University of Torino, Torino, Italy; [3] Tissue Engineering Group, Department of Histology, University of Granada, Granada, Spain; [4] Instituto de Investigación Biosanitaria Ibs.GRANADA, Granada, Spain; [5] IRCCS Istituto Ortopedico Galeazzi, Cell and Tissue Engineering Laboratory, Milan, Italy; [6] Regenerative Medicine Technologies Laboratory, Ente Ospedaliero Cantonale, Lugano, Switzerland; [7] Network for Transfusional Medicine, Cells and Tissues, Granada, Spain; * These authors have contributed equally to this work. Contact: ogarcia@ugr.es

Introduction: In critical nerve gaps where a direct tensionless repair cannot be applicable, an additional nerve graft or conduit is needed to fill the gap and connect the two transected nerve stumps [1]. Decellularized nerve allografts are considered promising tissue engineering strategies with superior regeneration results than nerve conduits [2]. These better results are related to the molecular composition and 3D organization of the extracellular matrix (ECM) obtained, but it is still necessary to improve these methods to obtain better outcomes. In the present work, we aimed to investigate a novel nerve decellularization protocol able to combine effective short-term decellularization with good preservation of the ECM component.
Methods: Two different decellularization protocols were tested. The first has proven to be efficient for tendons (DN-P1), consisting in 1 % tri (n-butyl) phosphate (TBP), 3 % peracetic acid (PAA) [3]. It was compared with a specifically developed decellularization protocol for nerves (DN-P2), using 125mM SB-10, 0.2% TritonX-100, 0.25% SDS, Sonification cycles [4]. The outcomes of both protocols were assessed by a series of in vitro evaluations, including qualitative and quantitative histological and immunohistochemical analyses, DNA quantification, SEM and TEM ultrastructural analyses, mechanical testing, and cell viability assay.
Results: Decellularization protocols tested had led to obtaining a well-preserved nerve-derived 3D ECM. DNA quantification showed that its content was significantly decreased, but not completely removed. Moreover, with both methods, it was not possible to completely remove the cell debris. DN-P1 showed better biomechanical and ultrastructural properties than those obtained with DN-P2. Similarly, better ex vivo biocompatibility was achieved with DN-P1 as compared to DN-P2.
Discussion: In this study, two novel decellularization methods were compared and promising properties were obtained ex vivo. From the structural, ultrastructural, and biocompatibility points of view superior results were obtained with DN-P1 than with DN-P2. However, it is necessary to optimize the method to ensure a more efficient cell removal. Furthermore, in vivo studies are needed to elucidate the therapeutic efficacy of these novel acellular nerve grafts.

Supported by the Spanish Plan Nacional de Investigación Cientifica, Desarrollo e Innovación Tecnológica (I+D+i) of the Spanish Ministry of Science and Innovation, grant FIS PI20/0318, Instituto de Salud Carlos III. Supported by Plan Andaluz de Investigación, Desarrollo e Innovación (PAIDI 2020), Consejería de Transformación Económica, Industria, Conocimiento y Universidades, Junta de Andalucía, Spain, grants P18-RT-5059 and A-CTS-498-UGR18 (proyectos de I+D+i en el marco del Programa Operativo FEDER Andalucía 2014-2020, University of Granada). Co-financed by the European Regional Development Fund (FEDER - ERDF).

[1] Carriel V., et al. (2014) Expert Rev Neurother. Mar;14(3):301-18
[2] Chato-Astrain J. et al. (2020) J Tissue Eng Regen Med. 2020 Jun;14(6):789-806
[3] Lovati A.B. et al. (2016) Stem Cells Int. 2016:7276150
[4] Boriani F., et al. (2017) J Biomed Mater Res A. Aug;105(8):2228-2240

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Wound healing modulation through the local application of powder collagen-derived treatments in an excisional cutaneous murine model

Benito-Martínez S.[1,2,3], Pérez-Köhler B.[1,2,3], Rodríguez M.[2,3,4], Izco J.M.[5], Recalde J.I.[5] and Pascual G.[1,2,3]

[1] Departamento de Medicina y Especialidades Médicas, Facultad de Medicina y Ciencias de la Salud, Universidad de Alcalá, Madrid, Spain; [2] Biomedical Networking Research Centre on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN, ISCIII), Madrid, Spain; [3] Ramón y Cajal Health Research Institute (IRYCIS), Madrid, Spain; [4] Departamento de Cirugía, Ciencias Médicas y Sociales, Facultad de Medicina y Ciencias de la Salud, Universidad de Alcalá, Madrid, Spain; [5] Viscofan S.A., Navarra, Spain.

Background: Wound healing includes dynamic processes grouped into three overlapping phases: inflammatory, proliferative, and maturation/remodeling. Collagen is a critical component of a healing wound and, due to its properties, is of great interest in regenerative medicine. This preclinical study was designed to compare the effects of a new collagen-based hydrolysate powder on wound repair to a commercial non-hydrolysate product (Catrix®), in a murine model of cutaneous healing.

Methods: Circular excisional defects (1.5 cm diameter) were created on the dorsal skin of Wistar rats (*n* = 36). Three study groups (*n* = 12 each) were established according to the treatment administered, being the third group a non-treated control. Treatments were applied at 0, 3, 5, 7 and 9 days. At the end of the established study times (7 and 18 days), the animals were euthanized in a CO_2 inhalation chamber.

To evaluate the wound closure, once the defect was performed and at the time of euthanasia, cenital photographs of the defects and scar tissue were taken. These measurements were used to calculate the relative values of the processes of wound closure, epithelialization, and contraction.

For morphological studies, hematoxylin–eosin, Masson's trichrome and Sirius red stainings were carried out. Hematoxylin–eosin and Masson's trichrome stainings allowed for the general observation of the repairing tissue, granulation tissue, distribution of collagen, inflammatory cells, and neoformed vessels. The Sirius red staining was utilized to evaluate the organization and maturation of collagen fibers in the repairing tissue. The data were expressed as the mean ± standard deviation. To compare different study groups, the Mann–Whitney U test was used. All statistical tests were performed using GraphPad Prism 5 software.

Results: The new collagen treatment led to the smallest open wound area throughout most of the study. After 7 days, wound morphometry, contraction, and epithelialization were similar in all groups. Treated animals showed reduced granulation tissue formation and fewer inflammatory cells, and induction of vasculature with respect to untreated animals. After 18 days, animals treated with the new collagen treatment showed accelerated wound closure, significantly increased epithelialization, and a more organized repair tissue.

Discussion and conclusions: Our findings suggest that the new collagen treatment, compared to the untreated control group, produces significantly faster wound closure and, at the same time, promotes a slight progression of the reparative process compared with the rest of the groups.

Supported by Viscofan S.A. (art. 83 LOU ref. 166/2017) and CIBER-BBN.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Gene expression of neomuscles generated by implantation of fresh and cryopreserved autologous adipose tissue in volumetric muscle loss

Leiva-Cepas F.[1,2,3,4], Jimena I.[1,2,3], Peña-Toledo M.A.[1,3,5], Agüera A.[1,2], López-Espejo M.E.[1,2], Martín-Hersog F.A.[3,6], Ruz-Caracuel I.[1,2,7], Villalba R.[3,8] and Peña-Amaro J.[1,2,3]

[1]Muscle Regeneration Group, University of Cordoba, Spain; [2]Department Morphological Sciences. Section of Histology, Faculty of Medicine and Nursing, University of Córdoba, Spain; [3]Maimonides Institute for Biomedical Research IMIBIC, Reina Sofia University Hospital; University of Cordoba, Cordoba, Spain; [4]Department of Pathology, Reina Sofia University Hospital, Cordoba, Spain; [5]Dementia and Multiple Sclerosis Unit, Neurology Service, Reina Sofia University Hospital, Cordoba, Spain; [6]Department Biochemistry and Molecular Biology, Faculty of Medicine and Nursing, University of Córdoba, Spain; [8]Center for Blood Transfusion, Tissues and Cells, Córdoba, Spain.

Background: Our group has shown that the implantation of fresh autologous adipose tissue allows reconstruction of volumetric muscle loss (VML) by generating new muscle tissue. This process takes place from the regenerative response of the edges of the lesion and the myogenic differentiation of resident stem cells in the adipose tissue. It is known that during the processes of muscle fiber regeneration and neoformation, different proteins involved in myogenic proliferation and differentiation, revascularization, remodeling of the extracellular matrix, among others, are co-expressed. The objective of the present study is to compare the gene expression of MyoD, IGF-1, collagen, laminin and VEGFA, in the neomuscles generated after implantation with fresh adipose tissue and with cryopreserved adipose tissue, in correlation with histological features.
Methods: Wistar rats were used and distributed into the following groups: i) Regenerative control group, consisting of rats injected i.m. with mepivacaine; ii) Group of rats that underwent an MLV in the tibialis anterior muscles and were implanted with fresh autologous adipose tissue (FAT: fresh adipose tissue); iii) Group of rats that had also suffered an MLV lesion that were implanted with autologous adipose tissue previously extracted and subjected to cryopreservation (CAT: cryopreserved adipose tissue). The animals were sacrificed, and the muscles removed at 14, 21, 28 and 60 days after the interventions. Samples were obtained and processed for microscopic study with histological, histochemical and immunohistochemical techniques. Others were processed for analysis of mRNA expression of genes encoding COL1A1, COL2A1, IGF1, MyoD1, VEGFA and laminin, by quantitative reverse transcription polymerase chain reaction (RT-qPCR).
Results: MyoD1 mRNA levels increased at 14 and 21 days in the FAT and CAT groups, stabilizing at 28 and 60 days in FAT and decreasing in CAT. The behaviour of VEGFA levels was similar to the behaviour of MyoD1, while IGF1 expression was decreased in the CAT group compared to the FAT group. Compared to the FAT group, the mRNA levels of COL1A1 and COL2A1 in the CAT group were especially elevated on days 21, 28 and 60. No significant differences in laminin expression were found when TAF and TAC are compared to the regenerative control.
Discussion and conclusion: Based on the results obtained, it seems that cryopreservation reduces the capacity of adipose tissue to generate new muscle tissue by reducing promyogenic factors and increasing profibrotic factors.

Acknowledgments: Supported by grant PS-2020-946 from Consejería de Salud y Familias, Junta de Andalucía, Spain.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Oleanolic acid anti-inflammatory and pro-angiogenic effects on endothelial cells obtained from umbilical cord of gestational diabetes affected women

Stelling-Férez J.[1,2], Pipino C.[3], Cappellacci I.[3], Di Tomo P.[3], Di Pietrantonio N.[3], Gabaldón J.A.[1], Pandolfi A.[3] and Nicolás F.J[2].

[1] Nutrition and Food Technology Department, Catholic University of Murcia (UCAM), Guadalupe, Murcia, Spain; [2] Regeneration, Molecular Oncology and TGF-ß Lab, IMIB-Arrixaca, El Palmar, Murcia, Spain; [3] Center for Advanced Studies and Technology (CAST), Department of Medical, Oral and Biotechnological Sciences, University G. d'Annunzio Chieti-Pescara, Chieti, Italy

Cutaneous wound healing is a regulated physiological process that involves several cell types. Among them, endothelial cells are required for inflammation resolution and neo-angiogenesis, both necessary for tissue restoration after injury. Primary human umbilical vein endothelial cells (HUVECs) are derived from umbilical cord, a perinatal tissue collected after delivery. When women develop gestational diabetes, chronic exposure to hyperglycemia induces in these cells (GD-HUVECs) epigenetic modifications leading to a permanent pro-inflammatory phenotype and impaired angiogenesis in contrast to control cells (C-HUVEC). Oleanolic acid (OA) is a bioactive triterpenoid known for its wound healing beneficial properties: epithelial cell migration stimulation and higher tensile strength of wounds. Because of its lipophilic nature, and to improve its aqueous solubility and stability, this molecule can be utilized if complexed in modified cyclodextrins (CD-HP-β). Not all OA benefits are disclosed, thus, the potential anti-inflammatory and pro-angiogenic properties of OA are still under investigation. We tested OA on C- and GD-HUVECs under inflammatory conditions induced by low levels of the inflammatory cytokine TNFα (1 ng/ml). A reduction of the expression of adhesion molecules, in particular Vascular cell adhesion molecule 1 (V-CAM1), was obtained after AO treatment. Coherently, and in response to TNFα, monocyte adhesion assays treated with OA showed a reduction in the number of adhered cells compared to untreated samples. What is more, OA and OA/CD-HP-β significantly stimulated angiogenesis in both C- and GD-HUVECs tube formation assays. Altogether, these results foresee OA potential as chronic wound healing agent, not just because of its positive role in cell migration, but also because of its capacity for inflammation resolution and angiogenesis promotion. In summary, these data anticipate a potential positive effect of OA in diabetic wounds.

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Secretome from macrophage increases major osteoclast-related markers in cultured osteoblasts

Toledano M.[1,2], Jacho D.[3], Osorio R.[1,2], Toledano-Osorio M.[1] and Yildirim-Ayan E.[3]

[1] Biomaterials Group, Department of Stomatology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs,GRANADA, Granada, Spain; [3] Bioengineering Department, University of Toledo, Toledo, Ohio, USA.

Introduction: Guided tissue/bone regeneration is necessary when treating periodontal and peri-implant diseases. Both have a multifactorial etiology with a clear infectious and immunologic component. An unfavorable imbalance between bone formation and resorption is produced, in the presence of periodontal pathogenic bacteria. An osteoconductive and osteoinductive environment will determine the success of the regenerative procedure of these diseases. Therefore, the interaction of osteoblasts and immune cells is of utmost importance. The objective of the present research was to ascertain the effect of activated M1 macrophage cytokine liberation on the activity of osteoblasts.

Methods: Cells from the human pro-monocytic cell line U937 (ATCC, Manassas, VA, USA) were encapsulated within 3 mg/ml collagen I solution and cultured in Roswell Park Memorial Institute (RPMI) 1640 medium. 3D cell-encapsulated collagen scaffolds were differentiated into macrophages (M0) and incubated in a standard cell culture incubator. After four days, PCR was performed on these cells to confirm M1 macrophage phenotype. After 7 days, macrophages culture media was collected and mixed (50%) with osteogenic media. Osteoblasts from an immortalized human fetal osteoblastic cell line (hFOB) were incubated in the presence or absence of macrophages conditioned media. Osteoblasts activity was assessed by alkaline phosphatase production. Two groups were used for the study: 1) hFOB ASC osteoblasts in osteogenic media and 2) hFOB CM where osteoblasts were cultured in osteogenic media and with the macrophages conditioning media. Alkaline phosphatase activity was once again evaluated. *RANKL*, *TRAF6*, *ACP5*, *NFkB*, *ALPL* and *CD206* expression were determined by means of PCR for both groups. Differences were assessed by Student t test ($p<0.05$).

Results: Main results are displayed in Figure 1.

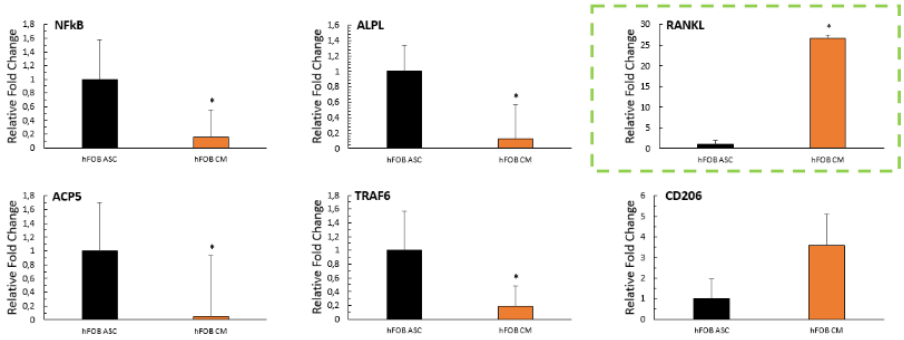


*Figure 1: Osteoclasts-related markers in osteoblasts cultured in the presence or absence of activated M1 macrophages conditioned medium (CM).*Indicates significant difference.*

Discussion & conclusions: Secretome from macrophages induced a decrease in alkaline phosphatase activity and increased major osteoclast-related markers. Therefore, the presence of activated M1 macrophages in periodontitis and peri-implantitis may produce a reduction on osteoblasts activity and favor the establishment of an environment promoting osteoclastogenesis.

Supported by Ministry of Economy and Competitiveness and European Regional Development Fund, grant number PID2020-114694RB-I00. M.T.-O. holds a FPU fellowship from the Ministry of Universities (FPU20/00450).

Histol Histopathol (2022) 37(S1)

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

POSTER PRESENTATION

Biomimetic mineralization of commercially available collagen membranes for bone regeneration

Osorio R.[1,2], Toledano-Osorio M.[1], Asady S.[1], Toledano M.[1,2] and Osorio E.[1,2]

[1] Biomaterials Group, Department of Stomatology, University of Granada, Granada, Spain; [2] Instituto de Investigación Biosanitaria ibs,GRANADA, Granada, Spain

Introduction: Guided bone regeneration is the most employed technique in order to achieve alveolar bone augmentation. A barrier membrane is used to prevent non-osteogenic cells from migrating into the bone defect, thereby enabling osteoprogenitors to grow at the defect area, exclusively. The most employed barriers are resorbable collagen membranes. Collagen membranes posse certain disadvantages, such as insufficient mechanical properties and poor dimensional stability overtime. In order to permit that collagen membranes have higher mechanical properties and remain longer at the wound, a process of biomineralization is desirable to happen. The objective of the present research was to ascertain differences in biomechanical properties and biomineralization between available collagen membranes from distinct origins.

Methods: Three collagen membranes were tested (1) Derma (porcine dermis); (2) Evolution Standard (porcine pericardium) and (3) Duo-Teck (equine) (OsteoBiol® by Tecnoss, Torino, ® by Tecnoss, Torino, Italy). Membranes were placed in sterile flasks containing 20 mL of simulated body fluid solution (SBFS) [pH 7.45] for 7 and 21 days at 37 °C, all experimental conditions were as specified at the ISO standard 23317:2014. Nanomechanical properties at each time point were assessed using the Hysitron Ti Premier nanoindenter (Hysitron, Inc., Minneapolis, MN) together with a commercial nano-DMA package. Differences in mechanical properties (complex modulus, storage modulus and tan delta) between membranes and time points were assessed, using the ANOVA test and Student-Newman-Keuls multiple comparisons ($p<0.05$). One half of each specimen was then fixed, subjected to critical point drying, sputter-coated with carbon and observed with a field emission scanning electron microscope (FESEM). Elemental analysis was also done by means of an energy dispersive analysis system (EDX) (Inca 300, Oxford Instrumts., Oxford, UK).

Results: Evolution was the membrane attaining the lowest mechanical properties ($p<0.05$), and no differences were found between Derma and Duo-Teck at initial time-point. Duo-Teck membrane did not withstand 1 week of immersion; therefore, mechanical values were not determined for any immersion time period. After 7 days of storage, the mechanical properties of Derma membrane increased (double-fold for Derma), but no differences were found between Derma and Evolution ($p=0.1$). After 21 days, Derma once again increased mechanical modulus (almost double-fold) ($p<0.05$). Evolution did not change in mechanical properties, overtime ($p>0.05$). Similar trend was encountered for storage modulus. Under FESEM, Duo-Teck presented a smooth surface and appeared as a corrugated layer, with somewhat oriented collagen fibers. At Duo-Teck surfaces, some agglomerates of particles were randomly distributed. Derma and Evolution showed a hierarchical 3D interconnected porous structure with a rough surface. Evolution and Derma were composed by collagen fibers of about 0.5 micron in diameter. At Derma surfaces, some other fibers were evidenced and distinguished from collagen due to their higher size (about 1 micron in diameter). In some specific zones, characteristic collagen fibers striation, showing crosslinking, was observed at Evolution and Derma membranes. Crosslinked fibers were much more abundant in Derma than in Evolution. The membranes microstructure was only slightly changed after 21 d. At Derma, a superficial mineral formation layer was evidenced on several areas. In some selected zones, at the periphery of the samples, mineralized fibers of more than 30 µm in diameter were encountered. After EDS, calcium and phosphate were detected in Derma surfaces.

Discussion & conclusions: The presence of elastin fibers in collagen membranes and a high crosslinking degree in Derma seems to favor mechanical properties of collagen membranes and/or biomineralization.

Supported by Ministry of Economy and Competitiveness and European Regional Development Fund, grant number PID2020-114694RB-I00. M.T.-O. holds a FPU fellowship from the Ministry of Universities (FPU20/00450).

Histology and Histopathology
From Cell Biology to Tissue Engineering

HISTOLOGY AND HISTOPATHOLOGY

F. Hernández, Editor
Professor of Cell Biology
University of Murcia
Murcia-Spain

M.T. Hernández, Manager
Murcia-Spain

J.F. Madrid, Editor
Professor of Histology
University of Murcia
Murcia-Spain

EDITORIAL BOARD:

This Journal is supported by

SOCIEDAD ESPAÑOLA DE HISTOLOGIA E INGENIERÍA TISULAR

UNIVERSITY OF MURCIA

University of Murcia

Indexed in: Science Citation Index, Science Citation Index Expanded (SciSearch), Journal Citation Reports/Science Edition, PubMed, Medline, SCOPUS, EMBASE, Chemical Abstracts Service (CAS), Google Scholar, EBSCO, CSA, CAB International, Biological Abstracts, BIOSIS, CAB Abstracts, Current Contents/ Life Sciences, SCImago, Zoological Record

Published by "Sercrisma International S.L." - Murcia, Spain

ISSN 0213-3911

Printed by "Jiménez Godoy, S.A." D.L. MU-33-1986 - Murcia, Spain