Histology and Histopathology

From Cell Biology to Tissue Engineering

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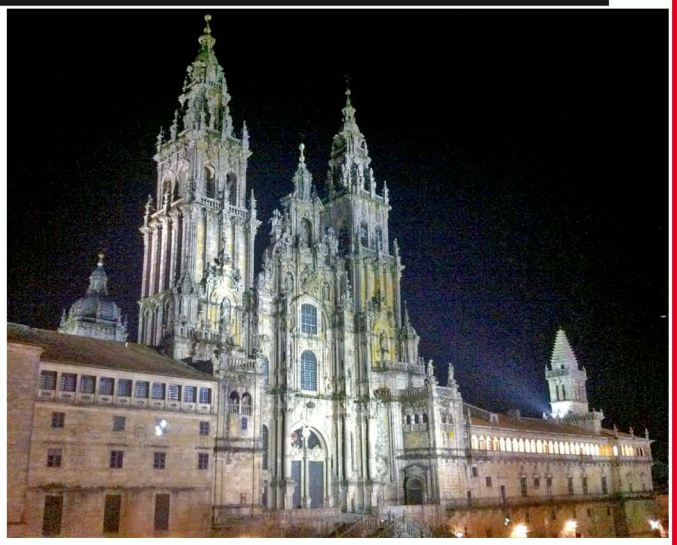
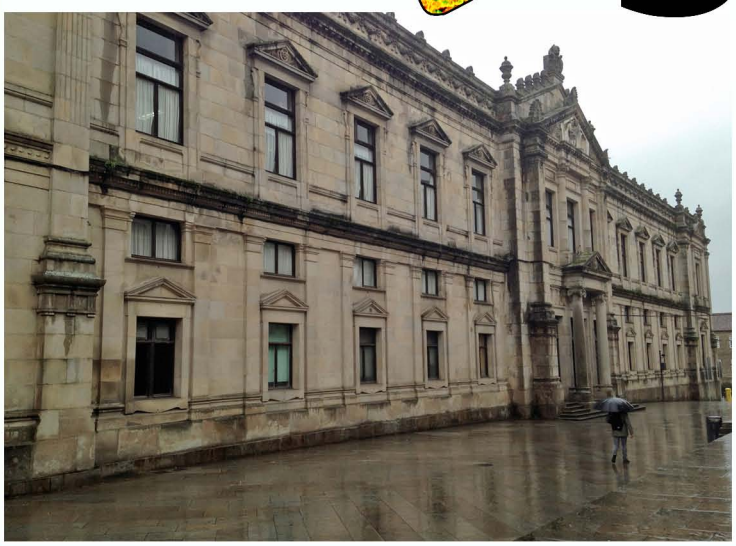


CONGRESO SEH/T



SANTIAGO DE COMPOSTELA

5 - 8 Septiembre 2017

XIX Congreso de la Sociedad Española de Histología e Ingeniería Tisular

IV Congreso Iberoamericano de Histología

VII Internacional Congress of Histology and Tissue Engineering

Santiago de Compostela, 5 – 8 de Septiembre de 2017

Honorary President

Andrés Beiras Iglesias

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PROGRAMA

XIX Congreso de la Sociedad Española de Histología e Ingeniería Tisular
IV Congreso Iberoamericano de Histología
VII International Congress of Histology and Tissue Engineering

5-8 de Septiembre 2017, Santiago de Compostela, España
Sede: Facultad de Medicina y Odontología, Universidad de Santiago

MARTES 5 SEPTIEMBRE

8:00 – 14:00

ÚLTIMA ETAPA DEL CAMINO DE FRANCÉS

8.30 AM SALIDA DESDE PLAZA GALICIA (TRASLADO EN AUTOBUS) LAVACOLLA - VAN MARCOS - RONTE DO GOZO- ZANTIAGO DE COMPOSTELA

16:00 – 21:00

CURSO PRECONGRESO

Salón de actos

- *Redacción y publicación de artículos científicos*

Juan Francisco Madrid, Francisco José Sáez (Univ. Murcia y País Vasco)

16:00 – 21:00

CURSO PRECONGRESO

Laboratorio de Histología

- *Inmunohistoquímica e Inmunofluorescencia*

Tomás García-Caballero, Rosalía Gallego, Ángel Vázquez-Boquete, Dora Ínsua, Roberto Montero Pazos (Univ. Santiago)

18:00 – 19:00

LA HISTOLOGÍA SE ABRE A LA SOCIEDAD

Aula Castelao

- *El proceso de la investigación microscópica en cáncer infantil: a la búsqueda de la curación.*

Rosa Noguera (Universidad de Valencia).

Entidades colaboradoras: NEN/Nicocontraelcáncerinfantil y AECC.

MIÉRCOLES 6 SEPTIEMBRE

8:30 Apertura secretaría técnica

9:00 – 14:00

CURSO PRECONGRESO

Salón de actos

- *Presentación pública de resultados científicos*

Juan Francisco Madrid, Francisco José Sáez (Univ. País Vasco)

9:00 – 14:00

CURSO PRECONGRESO

Aula de informática

- *Análisis de bioimagen microscópica*

Irene Tadeo, Rebeca Burgos, Pablo Vicente Munuera, Rosa Noguera (Univ. Valencia)

14:00 – 16:00

Comida

16:00

INAUGURACIÓN OFICIAL

16:30 – 17:00

CONFERENCIA INAUGURAL

Salón de actos

- *Senescencia celular: desde la fisiología a la patología*

Manuel Collado (CIMUS, Santiago)

17:00 – 17:30

CONFERENCIA

Salón de actos

- *La condrogénesis en la reparación del cartílago articular*

Francisco Blanco (INIBIC, A Coruña)

17:30 - 18:00

Café

18:00 – 18:30

PÓSTERES

18:30 – 19:30

COMUNICACIONES ORALES

19:30 – 20:00

CONFERENCIA

Salón de actos

- *Los nuevos retos de la histología odontológica*

Miguel Angel Marín (Univ. Granada)

20:00 – 20:30

CONFERENCIA

Salón de actos

- *Adrenomedulina: un péptido de mamíferos que actúa como un factor de crecimiento en plantas*

Alfredo Martínez (CIBIR, Logroño)

21:00

RUTA DE TAPAS

JUEVES
7 SEPTIEMBRE

8:30 Apertura secretaría técnica

9:00 – 9:30

CONFERENCIA

Salón de actos

- *Piel artificial. Del laboratorio a la clínica*

Miguel Alaminos, Víctor Carriel (Univ. Granada)

9:30 – 10:00

CONFERENCIA

Salón de actos

- *Pineal y melatonina: el poder y la fascinación de la oscuridad*

Eloy Redondo (Univ. Extremadura)

10:00 - 11:30

COMUNICACIONES ORALES

11:30 - 12:00

Café

12:00 - 12:30

PÓSTERES

12:30 – 13:00

CONFERENCIA

Salón de actos

- *La doble vida, conocida y oculta, de la célula de Leydig humana*

Javier Regadera (Univ. Autónoma Madrid)

13:00 – 13:30

CONFERENCIA

Salón de actos

- *Necesidad de los estudios histológicos en la búsqueda de mecanismos fisiopatológicos y farmacológicos*

Juan F. Padín (Univ. Autónoma Madrid)

14:00 – 16:00

Comida

16:00 – 16:30

CONFERENCIA

Salón de actos

- *Estudio morfofuncional del cilio primario en tiroides*

José C. Utrilla, José M. Fernández-Santos, Inés Martín-Lacave (Univ. Sevilla)

16:30 – 18:00

COMUNICACIONES ORALES

18:00 – 18:30

Café

18:30 – 19:00

PÓSTERES

19:00 – 19:30

CONFERENCIA

Salón de actos

- *Revistas "open access": ¿ángeles o demonios?*

Juan Francisco Madrid (Univ. Murcia)

19:30 – 20:30

ACTO HOMENAJE

PROFESOR ANDRÉS BEIRAS

Salón de grados

21:30

CENA

VIERNES
8 SEPTIEMBRE

8:30 Apertura secretaría técnica

9:00 – 9:30

CONFERENCIA

Salón de actos

- *Datación de traumatismos craneoencefálicos mediante técnicas inmunohistoquímicas*

Rosalía Gallego, Ángeles Romero, José Blanco-Pampín (Univ. Santiago)

9:30 – 11:00

COMUNICACIONES ORALES

11:00 – 11:30

Café

11:30 – 12:00

PÓSTERES

12:00 – 12:30

CONFERENCIA

Salón de actos

- *Empleo de diferentes recursos informáticos en la docencia de la histología*

Juan A. Pedrosa (Univ. Córdoba)

12:30 – 13:00

CONFERENCIA DE CLAUSURA

Salón de actos

- *Métodos de enseñanza de Histología / Biología celular en los Estados Unidos.*

Abraham L Kierszenbaum (The City University of New York)

13:00 – 14:00

REUNIÓN SOCIEDAD

14:00

CLAUSURA

ORAL COMUNICATIONS

AN ASSESSMENT OF THE MSCs ADMINISTRATION ROUTES IN SEPTIC RATS UNDERGOING HIGH VOLUME MECHANICAL VENTILATION.

Francisco Valladares Parrilla[1], Isabel García Laorden[2], Marta Martínez Cutillas[3], José Luis Carrasco Juan[3], Mª Nélida Rancel Torres[3], Ricardo Gutiérrez García[3], Lucio Díaz-Flores Feo[3]

[1]Department of Basic Medical Sciences. Section of Medicine. Faculty of Health Sciences. University of La Laguna. 38200 Tenerife. Spain. Networking Biomedical Research on Respiratory Diseases (CIBERES). Madrid. Spain.
[2]Research Unit. Gran Canaria University Hospital Dr Negrín. 35010 Las Palmas de GC. Networking Biomedical Research on Respiratory Diseases (CIBERES). Madrid. Spain.
[3]Department of Basic Medical Sciences. Section of Medicine. Faculty of Health Sciences. University of La Laguna. 38200 Tenerife. Spain.

Introduction: Mechanical ventilation is known to cause ventilator-induced lung injury (VILI) (inflammatory infiltrates, haemorrhage, atelectasis, and emphysematous areas); these events occur in different degrees depending on the state of health of the subject; thus, in cases with sepsis, the percentage of pulmonary parenchyma affected is greater than in the case of the healthy ones.

Material and Methods: Twenty-four animals were divided into four groups; prior to the administration of the cells, the animals were subjected to high volume mechanical ventilation (20ml/kg) for 4 hours; the first group (GIV) was intravenously injected with a solution rich in MSCs, while the second was given intratracheally (GIT), using the mechanical ventilator tube; control was performed with healthy animals (GOV and GOT); conventional light microscopy techniques (HE, Masson-Goldner and CD-117 stains) and observation were performed on a Nikon Optiphot-2 microscope.

Results: In the control groups, modifications consisted of atelectasis, emphysema and perivascular edema, with no difference between groups and MSCs. On the other hand, in septic animals, the observations showed morphological alterations similar, but of greater intensity, in both GIV and GIT groups; histological assessment using the IHQ technique of CD-117 revealed a higher proportion of MSCs in the interalveolar septum and within the pulmonary vessels themselves in the GIT with respect to GIV.

Conclusion: The most appropriate route of administration of MSCs to reach the lung is the intratracheal one.

Acknowledgments: CIBERES and Technicians of the Pathology Department of HUC and Basic Medical Sciences Department (Histology) of the ULL.

ROLE OF ECTO-NUCLEOTIDASES IN HUMAN ENDOMETRIAL PATHOLOGIES

Authors: *Mireia Martín-Satué[1,2], August Vidal[1,2,3], Aitor Rodríguez-Martínez[1,2], Carla Trapero[1,2], María Villamonte-Román[1], Mª Eulalia Fernández-Montolí[2,4], Josep Maria Piulats[2], Buenaventura Coroleu[5], Jordi Ponce[2,4], Xavier Matias-Guiu[2,3]*

Institution:

[1]*Departament de Patologia i Terapèutica Experimental, Facultat de Medicina i Ciències de la Salut, Campus Bellvitge, Universitat de Barcelona, Spain*

[2]*Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), Spain. CIBERONC*

[3]*Servei d'Anatomia Patològica, Hospital de Bellvitge, Barcelona, Spain*

[4]*Servei de Ginecologia, Hospital de Bellvitge, Barcelona, Spain*

[5]*Hospital Universitari Dexeus, Barcelona, Spain*

Introduction: Extracellular adenosine concentration in the tissue microenvironment increases during tissue stress conditions such as hypoxia, infection, metabolic stress, tumor transformation, and inflammation. High levels of extracellular adenosine have immunosuppressive and cell proliferation effects. The main source of adenosine is the dephosphorylation of ATP by ecto-nucleotidases. The aim of the present study was to characterize the expression and activity of ecto-nucleotidases in the context of two endometrial pathologies: endometrial cancer and endometriosis.

Material and methods: We analyzed, by means of immunolabeling and *in situ* activity assays, the following human endometrial tissues: 1) endometrial tumors and 2) eutopic endometria, as well as ectopic endometriotic lesions from women with endometriosis. For studies with tumors an *in silico* analysis was also performed. Moreover, endometrial cell lines were used to study the effects of different drugs, such as tamoxifen, known to increase the risk of developing endometrial cancer.

Results: Ecto-nucleotidases showed a differential pattern of expression in pathological conditions when compared with nonpathological endometria. This altered pattern included changes in expression levels and changes in protein localization, mainly a switch between epithelium and stroma. We have identified a novel mode of action of tamoxifen in endometrium that causes an increase in extracellular adenosine concentration.

Conclusions: Dysregulation of ecto-nucleotidase activity in endometrium might be the cause of endometrial pathologies with an inflammatory component such as cancer and endometriosis.

Acknowledgements: This work was supported by a grant from the *Instituto de Salud Carlos III* (*FIS PI15/00036*), co-funded by FEDER funds/European Regional Development Fund (ERDF)- "a Way to Build Europe"- // *FONDOS FEDER "una manera de hacer Europa",* and a grant from the *Fundación Merck Salud* (*Ayuda Merck de Investigación 2016-Fertilidad*). ARM was awarded a fellowship from the *Asociación Española Contra el Cáncer* (*AECC*). We are grateful for the technical support of Serveis Científics i Tecnològics, Campus Bellvitge, Universitat de Barcelona.

EXTRACELLULAR MATRIX GLYCOPROTEINS MECHANOBIOLOGY: MOLECULAR PLAYERS IN TUMOR SCAFFOLDING

Rebeca Burgos-Panadero[1], Irene Tadeo[1, 2], Irene Gimeno-LLuch[3], Mercedes Costell[3], Marcial García-Rojo[4], Samuel Navarro[1, 2]; Rosa Noguera[1, 2]

[1]Department of Pathology, Medical School, University of Valencia/ INCLIVA. Valencia, Spain. [2]CIBER of Cancer (CIBERONC), Madrid, Spain.[3] Department of Biochemistry and Molecular Biology, Faculty of Biological Sciences, University of Valencia, Valencia, Spain. [4]Department of Pathology, Hospital de Jerez de la Frontera, Jerez de la Frontera, Cádiz, Spain.

Introduction: The organization, composition and morfology of the extracellular matrix (ECM) components are key in healthy and pathological environments. Recent studies of our group have confirmed the influence of a stiff ECM with a poor outcome in neuroblastoma (NB). For better understanding of the architectural scaffolding of the ECM, we have applied digital image analysis to characterize fibronectin (FN) and vitronectin (VN) due to their adhesive role in tumor growth and metastasis. The objectives of this work are: 1) To detect and quantify the relationship between FN and VN, and 2) To establish their quantity and localization in a stiff ECM (stECM) versus a soft ECM (soECM) and their correlation between the area of vascular system.

Materials and methods: Sixteen NB were stained by immunofluorescence, using as primary antibodies anti-EDA (only detects the cellular FN, cFN), anti-FN (detects cellular and plasma forms of FN, pFN) and by immunohistochemistry to detect VN intercellular (iVN) (weak and moderate expression) and peri/intracellular VN (pVN) (strong expression). The images were digitalized at 40x and 20x respectively, using Pannoramic MIDI scanner (3D Histech) and quantified by Image Pro-Plus v.6.0 software (Media Cybernetics). We used an established ECM pattern obtained through logistic regression of morphometric parameters of ECM elements to define their association with clinical-biological risk factors in a cohort of 255 NB.

Results: The mean stained area of cFN was 2±2.9%, pFN was 16.8±17.5%, iVN was 12±11.5% and pVN was 2.80±3.86% in our NB cohort. We have found a positive correlation between i-pVN and cFN, and a negative correlation between i-pVN and pFN. In stECM, there were a great quantity of iVN, pVN, cFN and a low quantity of pFN. Finally, we have observed a negative/positive correlation between vascular elements and i-pVN/c-pFN, respectively.

Conclusions: The scaffolding and mechanical relationships between glycoproteins and other ECM elements can be defined using imaging technologies. The investigation of ECM components may be critical for a better understanding of tumoral behaviour and for the development of therapeutic strategies and diagnostic tools.

Acknowledgements: FAECC (2015/006), FIS (PI14/01008), RTICC (RD12/0036/0020) and CIBERONC (CB16/12/00484), Institute Carlos III, Madrid & ERDF.

M CELLS IN RABBIT APPENDIX: ITS AREA AND ORIGIN AFTER PARENTERAL ANTIGEN CHALLENGE

Roma, Stella Maris; Pérez, Fernando Adrián; D'Ottavio, Alberto Enrique

Department of Histology and Embryology. Medical School Research Council Rosario National University, Argentina

Introduction: Membranous (M) cells, identified in the gut follicle associated epithelium (FAE), are specialized in membranous traffic of luminal content to the immune cells of the lamina propia where the immune response initiates. Usually, rabbit M cells occupy 50% of the FAE area and its cytoplasmatic Vimentine may be specifically identified with anti-Vimentine (MAbs). This communication focuses in rabbit appendix FAE intending to make contributions on two currently unclear issues: (1) the response of M cells area to antigens parenterally administered, since existing experiments are centered in oral antigen challenges and (2) its origin (i.e: from enterocytes or from cryptal stem cells).

Material and Methods: Twenty animals were divided into four groups: Controls (G1); Complete and Incomplete Freund adjuvant –C and I FA- (G2); one subcutaneous injection of ovoalbumin (OVA) + CFA (G3) and two subcutaneous injections of OVA+CFA and OVA+IFA, respectively (G4). G2, G3 and G4 were euthanized 24 h after. In each group, slices were stained with anti-Vimentin (MAbs). FAE and M cells area was determined through image analysis software using optic microscopy. Other samples were used for Laser Scanning Microscopy.

Results: (1) FAE area G1: 2105±95.2 μ^2; G2: 1954±164.3 μ^2; G3: 1900±12.6 μ^2 and G4: 1945±49.3. These results were analyzed through Kruskal Wallis test considering significant $p<0.05$. The decrease in FAE area was statistically no significant and M cells occupied 50% of that in the four studied groups. (2) In FAE associated crypts, the first cells showing cytoplasmatic positivity to anti-Vimentine were those located in the so-called 7^{th} position. Likewise, it was detected higher expression in those cells localized in the slope of the crypt nearer to the follicle.

Conclusions: In all experimental rabbit groups: (1) Despite a discreet diminution in FAE area, the M cells percentage remained constant within its usual one unlike its increase in reported oral antigen challenged rabbits; (2) the cytoplasmic Vimentin expression pointed out that the M cells are predetermined before leaving the crypts, suggesting its stem cell origin.

EVIDENCE OF HYPOXIA IN PLACENTAL VILLI IN PREGNANT WOMEN WITH VENOUS INSUFFICIENCY

María J Álvarez-Rocha [1], Miguel A Ortega [1], Ángel Asúnsolo [2], Beatriz Romero [1], Juan De León-Luis [3], Melchor Álvarez-Mon[1, 4], Natalio García-Honduvilla [1,5], Julia Buján[1].

[1]Departments of Medicine and Medical Specialities. Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares, Madrid, Spain. Networking Biomedical Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain.[2]Department of Surgery, Medical and Social Sciences, Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares, Madrid, Spain.[3]Service of gynecology and obstetrics, Section of fetal maternal medicine, University Hospital Gregorio Marañón, Madrid, Spain. [4]Immune System Diseases-Rheumatology and Oncology Service, University Hospital Príncipe de Asturias, Alcalá de Henares, Madrid, Spain. [5]University Center of Defense of Madrid (CUD-ACD), Spain.

Introduction: Venous insufficiency affects 40-55 % of the world´s population. Lower extremity venous insufficiency (VI), usually with very late diagnosis occurs in 2-7 % of that group. The pregnancy period is a special moment for varicose diagnostics. VI is frequently reversible, even if a high percentage does not disappear after the childbirth or reappears in following pregnancies. In pregnant women VI can trigger a series of pathological consequences, which may affect placental cytoarchitecture.

Patients and Methods: A prospective cohort study was carried out on 67 pregnant women. A clinical history and exploration of lower limbs with the realization of Eco-Doppler to divide into two groups: those with or without VI using the Classification System for Chronic Venous Disorders (CEAP). After delivery, fragments of the placenta were taken and preserved for different studies. Optical and electron microscopy, Immunohistochemical and RT-qPCR.studies.

Results: Women with VI have shown an increase in placental hypoxia (Hif-1α), number of villi, pycnotic knots, bridges and apoptosis (Bax, Bcl-2, caspase 3 and 9) compared to the control group. We observed placental Tenney-Parker changes in pregnants with VI .

Conclusions: This situation shows clearly a remodelling of the placental tissues as a response to a hypoxic event which must be compensated. This remodeling implies changes at the placental barrier which are compatible with a loss of function which worsens the hypoxic state.

Acknowledgements: National Health Carlos III Institute (FIS-PI13/01513).

EPICORTICAL ROOTS AND SECONDARY HAUSTORIA OF *Struthanthus interruptus Kunth* (Loranthaceae)

Gabriela Hernández Maya[1], Carmen de la Paz Pérez Olvera[1], Jacqueline Ceja Romero[1]

[1]Biology departament. Universidad Autónoma Metropolitana, Unidad Iztapalapa, México City, Mexico

Introduction: Mistletoe are the plants that cause serious damages to the trees changing their development and morphology, reason why the host plants lose force and eventually they die. At UAM Iztapalapa there are trees parasitized by *S. interruptus*, from the Loranthaceae family, the anatomical description of the epicortical roots and secondary haustoria of this species are presented in this work in order to understand its structure. Due to the impact of parasitic plants on the trees, it is important to know their anatomical characteristics, for in the future propose methods of control.

Material and methods: Root and haustorial samples were obtained from tree branches of *Fraxinus uhdei* (Ash Tree). For the anatomical description, segments of the structures were placed in GAA, later they were included in paraffin and with a microtome of rotation were made transverse cuts and longitudinals of 25 µ of thickness, and they stained with Blue Astra - Fucsina basic, and mounted with entellant. The tissues were described and measured: pith, secondary xylem, secondary phloem, cortex and peridermis; the thickness of the wall and the diameter and length of the vascular elements were measured.

Results: The epicortical roots in their primary growth are polyarch, with a number of poles of protoxylem variable but usually more than 10, they have pith with a lot of starch; in secondary growth, the porosity is diffuse and the arrangement of vessels is radial and clustered, the radios are of one to three series, with vertical cells, the cortex has brachysclereids and the peridermis has more than 4 rows of cells. The secondary haustoria were originated from the epicortical root, anatomically can be seen laticiferous ducts.

Conclusions: The epicortical roots form secondary haustoria, which moreover brings support to the plant and helps them to obtain water. It is common to see secondary growth with vestiges of the primary. It is necessary to investigate the epicortical roots in mistletoes, because the sum of the morphological knowledge, physiological and particularly anatomical, can help to find control methods for this group of parasitic plants.

Acknowledgements: We thank the unit's gardening team for provide us the samples.

ASSESSMENT OF THE IMPORTANCE FACTOR IN TISSUE RESPONSES OF CLAM *POLYMESODA CAROLINIANA* EXPOSED TO CADMIUM

J. Ángel Vázquez Castro[1], Guadalupe Barrera Escorcia[2], Patricia Ramírez Romero[3], Irma Hernández Calderas[1], J. Roberto Jerónimo Juárez[1], Felipe de J. Muñoz González[1], Xochitl Guzmán García[1].

[1]Departamento de Hidrobiología. División de Ciencias Biológicas y de la Salud. Laboratorio de Ecotoxicología, Histopatología. Universidad Autónoma Metropolitana Unidad Iztapalapa. Ciudad de México, México.
[2]Departamento de Hidrobiología. División de Ciencias Biológicas y de la Salud. Laboratorio de Ecotoxicología, Biomarcadores. Universidad Autónoma Metropolitana Unidad Iztapalapa. Ciudad de México, México.
[3]Departamento de Hidrobiología. División de Ciencias Biológicas y de la Salud. Laboratorio de Ecotoxicología, Microbiología. Universidad Autónoma Metropolitana Unidad Iztapalapa. Ciudad de México, México.

Introduction: In international environmental monitoring, the use of histology as a biomarker has been proposed to evaluate biological responses in aquatic organisms subjected to stress. However, in the inferences of these analyzes, quantitative information derived from the observation of biological responses, which can be used in the estimation of health indexes in wild organisms or exposed to contaminants such as cadmium, is not considered. The objective of this work was to analyze the pathological importance factor of tissue responses observed in the Tecolutla's *Polymesoda clam,* exposed to cadmium.

Material and methods: Histopathological responses of 80 preparations of clams exposed to cadmium were analyzed. Fresh portions of the gonad and digestive gland were dissected. The preparations were analyzed by an optical microscope and the observed tissue responses were recorded. A prevalence matrix was constructed, integrating the main recorded alterations and their frequency of occurrence. The alterations were treated statistically and were related to the pathological importance factor (**IF**) of Cuevas (2013) to know the degree of tissue involvement.

Results: The main tissue responses observed were: brown cells, spherical inclusions, atrophies, cilia loss and eosinophilic secretions (in order of highest to lowest prevalence, respectively) in the digestive glands and gonads. Tissue responses were found mostly in organisms exposed to cadmium compared to controls. According to Cuevas, (2013) the tissue responses observed in the *P. caroliniana* clam have a **IF** of type 2, that is, "moderate reversible".

Conclusions: According to the IF, the alterations observed in this study do not compromise the health of the organisms. The use of categories of histopathological evaluation to establish a general health index in organisms exposed to cadmium is recommended.

Acknowledgements: To Biology Master of Universidad Autónoma Metropolitana. Unidad Iztapalapa. To the proyect "Indicadores de integridad Ecológica y Salud Ambiental".

ASSESSMENT OF THE STRESS BIOMARKERS IN THE CLAM *POLYMESODA CAROLINIANA* OF TECOLUTLA, VERACRUZ, MÉXICO

J. Roberto Jerónimo Juárez[1], Irma Hernández Calderas[1], María del R. Zarate Hernández[2], Marcela Arteaga Silva[3], Xochitl Guzmán García[1].

[1]Departamento de Hidrobiología. División de Ciencias Biológicas y de la Salud. Laboratorio de Ecotoxicología, Histopatología. Universidad Autónoma Metropolitana Unidad Iztapalapa. Ciudad de México, México.
[2]Departamento de Biología. División de Ciencias Biológicas y de la Salud. Laboratorio de Peces. Universidad Autónoma Metropolitana Unidad Iztapalapa. Ciudad de México, México.
[3]Departamento de Biología de la Reproducción. División de Ciencias Biológicas y de la Salud. Laboratorio de Neuroendocrinología. Universidad Autónoma Metropolitana Unidad Iztapalapa. Ciudad de México, México.

Introduction: Environmental stress caused by human activities may lead to alterations in the physiological status of aquatic organisms such as bivalve molluscs. Markers of biological responses like immunohistochemical analysis of HSP70, Metallothioneins and Cytochrome P450 have been proposed as a tool to evaluate stress by pollution. In Mexico, there are areas such as Tecolutla Veracruz, where strategies are required to evaluate the physiological status of the organisms. The aim of this work was to evaluate biomarkers related to environmental stress and the presence of hydrocarbons in the *P.caroliniana* clams of Tecolutla, Veracruz.

Materials and methods: Clam tissue samples from Tecolutla, Veracruz were analyzed. Tissues were processed by the histological technique and 5 micron cuts were made in the middle region of the body. The sections were deparaffinized and rehydrated. Antigen retrieval was made by pressure cooker. Endogenous peroxidases was neutralized by incubating and the monoclonal antibodies were applied for HSP70, Cyt P450, and Metallothionein, as well as the secondary antibody. It was used DAB+Substrate Buffer DakoCytomation as a detection system and DAB+Chromogen Dako. The sections were counterstained with Tacha´s Hematoxylin and were observed under an optical microscope.

Results: The immunolocalization of specific markers was observed in the cytoplasm and some cell nuclei of epithelia in the digestive tract. Positive immunoreaction of HSP70 suggests the induction of antiapoptotic functions or tissue repair. Metallothioneins suggests the presence of metals in the environment where they develop. P450 overexpression was positive in gill epithelia; indicating the presence of hydrocarbons. The tissue structure that showed greater sensitivity to the markers was the foot, where it was possible to observe cells with granular content, reported previously as brown cells or hemocytes, which participate in the mechanisms of detoxification and its manifested in the positive response of the biomarkers used.

Conclusions: The overexpression of environmental stress markers involves the activation of biochemical responses in the clam P. caroliniana. Proteins that are overexpressed against different sources of pollution, represent a potential biomarker in the evaluation of environmental stress.

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USE OF THREE DIFFERENT SOLVENT MIXTURES, FOR THE PROCESS OF PARAFFIN ROUTINE INCLUSION APPLIED IN VEGETABLES.

Garrido-Fariña German I[1].

[1]Laboratorio de Apoyo a Histología y Biología, Departamento de Ciencias Biológicas, Facultad de Estudios Superiores Cuautitlán, Universidad Nacional Autónoma de México, Km. 2.5 Carretera Cuautitlán-Teoloyucan, San Sebastián Xhala Cuautitlán Izcalli, Edo de Méx CP 54740. México. e-mail: isaurogafa@yahoo.com.mx

INTRODUCTION: In the routine paraffin inclusion technique (TIPR), the alcohol is replaced with solvent, enable the tissue clarifying and allowing paraffin infiltration. The intermediate or "lightening" reagents must be miscible with alcohol and dissolve the paraffin. Intermediate reagents have advantages and disadvantages, but there is no ideal reagent. This work proposes the use of three solvent mixtures as an intermediate reagent to improve the working conditions during the TIPR using two routine fixatives for vegetable material.

MATERIAL AND METHODS: In the LAHB of the UNAM, mixtures of: Xylene-Benzene (XB), Xylene-Toluene (XT) and Xylene-Chloroform (XCL) have been routinely used in animal material. *Elodea canadensis* (André Michaux, 1787) samples, were fixed with the formaldehyde mixtures (FAA) and Craft, processed by the TIPR, during the rinse stage, were applied: styrene monomer, XCL, XB and XT. The blocks were cut to 12 µm thick, and stained with safranin-solid red. During cutting, the following characteristics were observed in the block: hardness or shear strength, tissue friability, shear elasticity, hygroscopicity or hydratability, cut resistance and cut distention. The duration of the smell of solvents in the block was appreciated over 7 and 14 days.

RESULTS: The clearing time for the three mixtures was: XB 40-50 min, XT 40-60 min and XCL 20-40 min, observing higher speed in the last mixture. The odor of the XCL mixture was not perceptible, it provokes the best conditions for its processing with any fixation, more homogenous behavior facilitating cutting and mounting.

CONCLUSION: The process of clarification is a fundamental step in order to obtain an adequate result. There are no recommendations in the literature for the use of solvent mixtures. Blends can aid in the processing of complicated samples. The XCL increases the rate of infiltration and clearing perception, leave no traces of alcohol, allow rapid infiltration, softens difficult samples such as neoplasms or with different densities, can remain overnight without causing damage or difficulties for the process. On the other hand, dyeing capacities are not modified.

HISTOLOGICAL DESCRIPTION OF THE EYE DURING THE EMBRYONIC DEVELOPMENT OF THE PACIFIC PYGMY OCTOPUS *Paroctopus digueti* (PERRIER & ROCHEBURNE, 1984)

Maritza García-Flores[1], Alma R. Rivera-Camacho[1], Marcial Arellano-Martínez[1], Bertha P. Ceballos-Vázquez[1].

[1]Departament of Marine Biology and Fisheries. Marine Invertebrate Biology Laboratory. Interdisciplinary Center for Marine Sciences. Instituto Politécnico Nacional. La Paz, B.C.S. México.

Introduction: Paroctopus digueti is a small size specie (15 cm), with parental care y direct development, it produces bentonic oganisms what makes posible its breeding in laboratory conditions since egg to juvenile, but knoledge of its embryonic development still necesary. The eye development (lens, retina and optic lobes) in P. digueti embryo were studied historicly since early fases through its eclosion.

Material and Methods: Females with eggs were kept in a close recirculating system with mechanical, biological filters and UV at a 27± 1 temperature with constant aireation. The morphologycal identification of the 33 phases of embryonic development was performed looking at the degree of develope of fune, eyes, arms and chromatophores. The eggs were fixed in formaldehyde (5%) with a pots-fixed with Davidson's solution for 24 hours.Traditional histologyc process was performed with Gomori´s trichromic stain.

Results: In phase IX differentiation of retina of nuclear support cells and proximal segment of photoreception cells was observed. In optical lobe development of the medular neuropil, starting with the separation of the nerve cells (phase IX), its complete development was observed in phase XV. The diferentiation of the photoreceptor cells distal segment was observed in phase XIII with a dark brown pigmentation. Ciliar boddies were clearly observed in phase XX. Iris is found in phase XXIII upper the ciliar boddies. Lens presents completely develop in phase XXVII. Nerborns clearly presents all the structures.

Conclusions: This research constitutes the first description of the eye at a histological level during the embryonic development of P. digueti.

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ACTOMYOSIN DYNAMICS DRIVEN BY BMP2 SIGNALING PATHWAY UNDERLIE THE EMERGENCE APLICAL EXTRUSION OF THE PROEPICARDIUM IN ZABRAFISH

Laura Andrés-Delgado[1,2], David Bazaga[1], María Galardi-Castilla[1], Nadia Mercader[1,3]

[1]Development of the epicardium and its role during regeneration laboratory, Centro Nacional de Investigaciones Cardiovasculares Carlos III, Melchor Fernández Almagro 3, 28029 Madrid, Spain.
[2]Departamento de Anatomía, Histología y Neurociencia, Facultad de Medicina, Universidad Autónoma de Madrid, Spain.
[3]Institute of Anatomy, University of Bern, 3000 Bern 9, Switzerland.

Introduction: The epicardium, the outer layer enclosing the myocardium, plays key roles in heart development and regeneration. And also contributes to progenitor cells for the coronary vasculature and intracardiac fibroblasts. During embryogenesis, it arises from the proepicardium (PE), a cell cluster that appears in the dorsal mesothelial pericardial tissue in between the venous pole and the atrioventricular canal boundary region of the heart. Little was known about how the PE emerges from the pericardial mesothelium.

Material and methods: Using the zebrafish model we combined the use of genetic tools, pharmacological treatments, *in vivo* imaging with cellular resolution and computational approaches to manipulate signaling pathways, the heartbeat and the actomyosin cytoskeleton dynamics.

Results: We have developed a new method to quantify pericardial cell movements occurring prior to PE formation. We showed that a coordinated collective movement of the dorsal pericardium drives PE cluster formation and PE cells are lately apically extruded.
Interestingly, we founded that the treatment with the Myosin inhibitor 2,3Butanedione Monoxime (BDM), reverted PE formation in a process whereby PE cells get flattened and regress, which means that an intact actomyosin cytoskeleton is necessary for this process.
Furthermore, we showed that Bmp pathway controls the actin cytoskeleton dynamics in pericardial mesothelium, and could rescue the formation of PE cluster under actomyosin inhibition.

Conclusions: Our results revealed that the coordinated action of Bmp signaling and actomyosin mediated tissue tension play a fundamental role in mesothelial PE formation. We provided evidence that apical extrusion, a cellular behavior studied during epithelial tissue homeostasis and cancer also operates during embryonic morphogenesis.

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STUDY HISTOLOGICAL AND HISTOCHEMICAL OF THE MANTLE OF *MODIOLUS CAPAX* (CONRAD, 1837) BIVALVIA MYTILIDAE

Esther Uría-Galicia[1], Susana Hernandez-Blanco[1]

[1]Departament of Morphology, Laboratory of Histology Animal, Nacional School of Biological Sciences National Politecnic Institute, México City, México.

Introduction: *Modiolus capax* is consumed locally and used as a biomonitoring of aquatic pollution. The morphological characteristics of any organ, such as the structure of the mantle, are important for ecological studies, systematic and cladistic analysis. Histochemical studies of this kind are scarce, so the goal is perform histological and histochemical analysis of the mantle to determine its structure as a basis for future studies, such as the formation of the shell and pollution monitoring.

Material and Methods: Six specimens were collected in the Bay of La Paz, BCS, México. Mantle was dissected and divided into six regions, fixed in 10% formaldehyde in seawater, embedded in paraffin and freezing and staining techniques for histological analysis, and histochemical for particular components were performed.

Results: The molluscan mantle organ forms the outer integument that encloses the body of animal, their two lobes present epithelium: Simple ciliated columnar, simple ciliated cubic and stratified ciliated columnar. The mantle margin is the free edge, this consist of three folds: In the outer and middle, interepithelial and subepithelial glands are mixed, Alcian blue and PAS positive. Between both folds is the periostracal groove where the periostracum is produced, which together with glandular secretions are involved in the development of the shell. Smooth muscle bundles have multiple orientations and are most abundant in the inner fold, regulating the streams and sealing the pallial cavity. The connective tissue of the mantle zone presents reproductive tissue and cells containing lipids and glycogen used primarily gametogenesis. Traces of Fe, but not Cu, due to the bioregulation mechanism of these metals, were found.

Conclusions: The epithelium of the marginal zone of the mantle has similarities with other bivalves, such as *Mytilus edulis, Pinctada margaritifera* and *Pinctada mazatlanica.* The types of glands are the same, but differ with *M. capax* in the proportion, location and chemical composition, mainly of acid glycoproteins. *M. capax* presents more quantity of smooth muscle tissue bundles in the inner fold coinciding with *P. margaritifera* and *P.mazatlanica.* In *P. mazatlanica* glycogen is found only in the mantle epithelium, and in *M. capax is in* the connective tissue cells, in the pallial zone and the epithelia of the outer and middle lobes.

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TESTIS AND EPIDIDYMIS OF WISTAR RATS AFTER SEXUAL SATIATION: HISTOMORPHOMETRIC ANALYSIS

García-Lorenzana Mario[1], Tlachi-López José Luis[1], Arenas-Ríos Edith[1] López-Ramírez Yolanda[1], Verónica Rodríguez Piedracruz[2] and Lucio Rosa Angélica[2]

[1]Departamento de Biología de la Reproducción, Universidad Autónoma Metropolitana, Ciudad de México. [2]Centro Tlaxcala de Biología de la Conducta, Universidad Autónoma de Tlaxcala, Tlaxcala, Tlax., México. (mglo@xanum.uam.mx)

Introduction: Sexual satiety is the inhibition of copulation due to repeated mating. It is recognized when the male does not display mounts neither motor pattern of intromissions during 30 minutes after the last ejaculatory series. It is assumed that sexually satiated males under the Coolidge effect resume copulation until ejaculation. However, we evidenced that males execute the ejaculation pattern without seminal expulsion. Also we described that sexual satiation produce qualitative histological changes in the epididymis and testis. Finally, we showed a gradually recovery in the ejaculate and in the capacity to induce pregnancy. Therefore, we decided to describe the histological and morphometric features of the testis and epididymis, and to analyze the characteristics of the sperm obtained from testis and epididymis at different post satiety days.

Material and Methods: Twenty-seven sexually experienced male rats were housing under standard conditions. After sexual satiety tests they were sacrificed bioethically (NOM-062-ZOO-1999) at 4, 8, 12, 16, 20, 24, and 28 days (d). Also, three experienced but non-sexually satiated males were considered as a control group (C). Left or right organs were fixed with Bouin-Duboscq, and processed using the routine histological techniques. Hematoxylin-Eosin was used to stain tissue sections. Testis parameters were: 1) number of cellular associations of the seminiferous cycle, 2) germinal epithelium area of the seminiferous tubules, and 3) Leydig cell density. Epididymis parameters were: 4) sperm density, 5) types of cellular epithelium 6) duct area, and 7) sperm parameters (sperm motitity, sperm count, sperm viability). U Mann-Whitney was used for morphometry and cell type analysis, and ANOVA followed by Tukey-Kramer for epithelial type and sperm parameters.

Results: Changes in all parameters were observed particularly at 4d, 8d and 12d; a recovery was detected at 16d.

Conclusions: Results may be due to changes in androgens concentration, androgen receptor density, enzymatic activity.

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NEONATAL HYPOXIA-ISCHEMIA REDUCES NEUROGENESIS IN THE SUBVENTRICULAR ZONE OF THE NEWBORN PIGLET: POSSIBLE ATTENUATION BY THERAPEUTIC HYPOTHERMIA

Daniel Alonso-Alconada[1,2], Pierre Gressens[3,4,5,6], Nicola J Robertson[1].

[1]Institute for Women's Health, University College London, London, UK.
[2]Department of Cell Biology and Histology, School of Medicine and Nursing, University of the Basque Country, Leioa, Bizkaia, Spain.
[3]Department of Perinatal Imaging and Health, Division of Imaging Sciences and Biomedical Engineering, King's College London, King's Health Partners, St. Thomas' Hospital, London, UK.
[4]Inserm, U1141, Paris, France.
[5]University Paris Diderot, Sorbonne Paris Cite´, Paris, France.
[6]PremUP, Paris, France.

Introduction: Intrapartum-related insults at full term such as hypoxia-ischaemia (HI) are the third leading cause of global child deaths. Neuroprotection combined with neuroregeneration may be critical for optimizing functional recovery in neonatal encephalopathy. Using a piglet model of neonatal asphyxia, the aim of the present work was to investigate the influence of HI on the subventricular zone (SVZ) neurogenic niche and its modulation by therapeutic hypothermia.

Material and methods: After transient HI (occlusion of both common-carotid arteries + FiO_2 reduction to 6% vol/vol), 34 newborn piglets (<24h) were randomized to: normothermia (38ºC) or three different hypothermic target temperatures by whole body cooling to 35ºC, 33.5ºC, or 30ºC during 2-26 h (all n=7). Non-hypoxic-ischemic animals (Naive, n=6) served as controls. At 48h, piglets were euthanized and SVZ obtained for histology and immunohistochemistry of doublecortin (DCX, a marker of immature migrating neuroblasts), Ki67 (cell proliferation) and Ki67+Sox2 double labelling (neural stem/progenitor cells).

Results: Normothermic animals showed a reduction ($p<0,05$) in the area of neuroblast chains and DCX immunofluorescence, an effect reverted only with cooling to 33.5ºC. HI also induced a decrease ($p<0,05$) in cell proliferation and Ki67+Sox2 double positive cells, which was avoided with cooling to any of the target temperatures.

Conclusions: Neonatal HI induces a decrease in cell proliferation and neurogenesis in the SVZ of the newborn piglet which can be attenuated by therapeutic hypothermia.

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HISTOLOGICAL STUDY OF THE THERAPEUTIC EFFECT OF CANNABINOIDS AFTER NEONATAL HYPOXIC-ISCHEMIC BRAIN INJURY

Daniel Alonso-Alconada[1,2], Enrique Hilario[1], Francisco J. Álvarez-Díaz[3], Antonia Álvarez A[1].

[1] Department of Cell Biology and Histology, School of Medicine and Nursing, University of the Basque Country, Leioa, Bizkaia, Spain.
[2] Institute for Women's Health, University College London, London, UK.
[3]Intensive Care Translational Research Group, Biocruces Health Research Institute, Bizkaia, Spain.

Introduction: Perinatal asphyxia-induced brain injury is a common form of neonatal brain damage and one of the major causes of developmental disability in children. Cannabinoids can modulate the intensity and extension of neurotoxic processes, the inflammatory response and cell death or survival in a variety of pathological conditions, thus emerging as a pot ential neurotherapeutic for neonatal encephalopathy.

Material and methods: We used a fetal lamb model of perinatal asphyxia that mimics the clinical situation of asphyxiated neonates, in which the decrease in blood flow and hypoxia are generally due to an um bilical cord compression. Animals were assigned to: one sham group (sham) and two hypoxic-ischemic (HI) groups that received a dose of 0.01 ug/kg of the cannabinoid agonist WIN55,212-2 (HI+WIN) or vehicle (HI+VEH) after 60 min of partial occlusion of the umbilical cord, and then sacrificed 3 h later. Different brain regions were separated for morphological studies and the same territories were dissociated and analyzed by flow cytometry to evaluate the pattern of brain damage and the inflammatory response.

Results: After HI, substantial brain injury was found in HI+VEH group and a clear reduction when the cannabinoid agonist was administered. The histological evaluation of the presence of TNF-alpha, IL-1B, and IL-6 in both sham and HI+WIN animals revealed that these brains did not display immunoreactivity for these cytokines. Flow cytometry quantification showed that TNF-alpha, IL-1B and IL-6 levels were reduced ($p<0,05$) in cortex, basal nuclei, hypothalamus, thalamus, hippocampus and cerebellum after WIN treatment.

Conclusions: Our results suggest that the cannabinoid agonist WIN 55,212-2 reduced the early inflammatory response induced by hypoxia-ischemia in a f etal lamb model of neonatal asphyxia.

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TRPC1 CALCIUM CHANNEL IN HEALTHY RETINAS AND DURING NEURONAL DEGENERATION

Elena Caminos, Mercedes I. Jaquero, Juan R. Martinez-Galan

University of Castilla-La Macha. Faculty of Medicine in Albacete. Dpto. of Medical Sciences. Instituto de Investigación en Discapacidades Neurológicas (IDINE). Albacete. Spain.

Introduction. TRPC1 (transient receptor potential canonical 1) is a plasma membrane calcium channel that is activated by metabotropic glutamate receptors stimulation. TRPC1 has been isolated from retinal cell cultures where it modulates calcium concentration. High intracellular calcium levels can lead to cell death during degenerative process such as Retinitis Pigmentosa (RP). Thus, the study of channels that affect calcium concentrations will be important to better understand these pathologies and develop possible therapies. The objectives of this study were to characterize TRPC1 localization in healthy rat retinas and to detect modifications of its expression in diseased rat retinas with RP.

Material and Methods. Retinas from wild-type Sprague-Dawley rats and P23H-1 transgenic rats as a model of RP, on postnatal days 20, 90 and 150, were used in our experiments. *In toto* and *in situ* retinas were stained by double immunofluorescence cytochemical techniques using combinations of primary antibodies.

Results. We detected TRCP1 expression mainly into postsynaptic dendritic areas in the inner plexiform layer, but it was also detected in the somata of some cells in the inner part of the inner nuclear layer and in pigment epithelium cells. Higher intensity of TRPC1 labeling was found in P23H-1 during neuronal degeneration. TRPC1/MAP-2 colocalization increased during the degenerative processes, while unappreciated double label was detected in presynaptic terminals. In addition, there were changes in the localization of TRPC1 immunoreactivity in the pigment epithelium cells of retinas with RP.

Conclusions. This higher expression of TRPC1 during degeneration suggests that the channel could contribute to typical cellular destruction in these types of pathologies by altering calcium homeostasis and, on the other hand, it could be a mediator of the neuroprotective effect from pigment epithelium cells through glutamate secretion. It is therefore logical to conclude that TRPC1 probably plays a specific role during degenerative processes such as RP and that future works will be necessary to understand its implication in the disease.

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NEUROHISTOLOGY OF THE INTERTHALAMIC ADHESION IN HUMAN

Jorge Eduardo Duque Parra [1,2]. John Barco Rios [1]. Alejandro Vera [1]. Genaro Morales Parra [2]

1 Departamento de Ciencias Básicas, Facultad de Ciencias para la Salud, Programa de Medicina, Universidad de Caldas, Colombia.
2 Departamento de Ciencias Básicas, Facultad de Ciencias para la Salud, Programa de Medicina, Universidad de Manizales, Colombia.

Introduction: the interthalamic adhesion, or *massa intermedia* (MI), is a neuroanatomical structure of the diencephalon. The MI is interposed in the third ventricle and its formed by a fusion between the right and the left parts of the thalamus. For some authors, the interthalamic adhesion is a gray commissure forming a bridge of tissue across the midline, for others, it has commissural fibers and it does not contain any neuron cell. Histologically, in the human adult various types of neurons have been identified inside the MI such as fusiform, triangular, multipolar, and oval neurons. A greater frequency of absence of the MI in males has been reported, and its presence has been associated with behavioral disorders in psychiatric patients. The presence of a MI could affect the pattern of cerebrospinal fluid pressure distribution therefore it has been associated with development of hydrocephalus. Despite its anatomical and physiological importance, the MI remains poorly studied and the clinical significance of its presence or absence remains largely misunderstood.

Material and Methods: Nine brains of adult men in which interthalamic adhesion was observed were used in this study. Interthalamic adhesion was exposed through a middle line incision above the corpus callosum. Photographic records were taken and histological processing was performed by hematoxylineosin staining.

Results: The interthalamic adhesions studied had few bodies of neurons, with pyramidal, circular and oval shapes. These neurons were aligned in the anteroposterior direction, but not in the direction of the thalamus, as might be expected in the case of a commissure. Small size glial cells were also identified. It was found that the minority of the nervous tissue were neuron bodies.

Conclusion: These observations don´t support the role of the interthalamic adhesion in humans as a gray commissure, and shows that the MI contains neuroglia and some bodies of neurons.

REPRODUCTIVE STUDY OF THE RED CLAM *Megapitaria aurantiaca* (BIVALVIA: VENERIDAE) IN PUERTO LIBERTAD, GULF OF CALIFORNIA, MEXICO, WITHIN THE FRAMEWORK OF A MANAGEMENT PLAN.

Romo-Piñera, A.[1] y Hernández-Moreno, E.[1]
[1]Universidad Autónoma de Baja California Sur. Departamento Académico de Ciencias Marinas y Costeras. Carretera al Sur Km 5.5. La Paz, B.C.S. 23088, Mexico.

Introduction: The study of the reproductive biology of species of commercial interest, generates basic and indispensable information that allows the development of sustainable fisheries. In Mexico, the fishing resource "clam", is of great interest due to the economic spill that it represents. The red clam, *Megapitaria aurantiaca,* is within this resource, by which in the state of Sonora has increased the commercial interest, this due to the decreased populations or times of no-catch present for other species.

Material and Methods: In this study we analyzed the reproductive cycle and sexual ratio of *M. aurantiaca*, as well as their size of first maturity. From March 2015 to March 2016, an average of 30 clams was collected in Puerto Libertad, Gulf of California. Their length, total weight and weight without shell were taken from each clam. The gonadal tissues were histologically processed and stained with Gill´s Hematoxylin-Eosin-Floxin protocol.

Results: A total of 375 clams were analyzed, represented by 149 females and 177 males. The sexual ratio was of 1F: 1M ($p < 0.05$). Four stages of gonadal development (gametogenesis, mature, spawned, post-spawned) were identified. Organisms in gametogenesis were presented throughout the year in both sexes. Mature males were observed from March to August (2015) and February and March (2016), while mature females were observed from March (2015) to November (2015) and February (2016). The emission of spermatozoids was present from April to June and August. The spawned female was present from April to June and August. The undifferentiated correspond to organisms not recruited to reproduction with length of 46 to 114 mm. Females and males in development were observed from 64 and 67 mm of length, respectively.

Conclusions: The red clam *M. aurantiaca*, has a spawning period from March to August, with reproductively active organisms starting at 64 mm of length. Therefore, a not-catch from March to August and a legal catch size of 68 mm of lengt, could be considered.

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PROLIFERATION AND APOPTOSIS OF SERTOLI CELLS IN THE SYRIAN HAMSTER (*Mesocricetus auratus*) DURING RECRUDESCENCE AFTER EXPOSURE TO SHORT PHOTOPERIOD

Martínez-Hernández, J.[1], Seco-Rovira, V.[1], Beltran-Frutos, E.[1], Ferrer C.[1], Canteras M.[2], Pastor L.M.[1]

[1]Department of Cell Biology and Histology School of Medicine, IMIB-Arrixaca. Regional Campus of International Excellence. Campus Mare Nostrum. University of Murcia. (30100).
[2]Department of Statistics. School of Medicine, University of Murcia. (30100).

Introduction: The Syrian hamster is a seasonal-breeding rodent that presents two well-defined stages in its reproductive cycle regression and recrudescence. The Sertoli cell (Sc) is known for its support function in spermatogenesis and has been described as a quiescent cell once the animal has reached sexual maturity. Recent studies have described the proliferation of Scs and also their loss through apoptosis in several species of rodent. The objectives of the present study were to determine proliferation (PI) and apoptosis (AI) indices as well as any variation of the total number of Scs during testicular recrudescence after exposure to a short photoperiod.

Material & Methods: For this, a total of 24 hamsters were used (18 treated and 6 controls (CT)). The treated animals were subjected to an 8:16 light-dark photoperiod, while the control animals were subjected to a 12:12 light-dark photoperiod. Six treated animals plus two from the control group were sacrificed at 16, 19 and 21 weeks. Testes were fixed in methacarn. Three recrudescence groups were established: Initial (IR), Advanced (AR) and Total (TR) recrudescence. Histological sections of testes were submitted to: H&E, TUNEL, vimentin immunohistochemistry and double staining protocols for dark field microscopy (Vimentin-PCNA and TUNEL-Vimentin immunofluorescence). For each group, the PI, AI and total number of Scs were studied by points method.

Results: The results revealed the existence of Vimentin +/TUNEL + as well as Vimentin +/PCNA + cells. The total number of Sc increased between IR and AR, reaching values similar to the controls. PI was significantly higher in RI than in the other groups. AI did not vary significantly between the recrudescence groups compared to control.

Conclusion: a) fluorescence microscopy revealed the proliferation and apoptosis of Sc in all the groups studied; b) the PI began high in IR and gradually decreased to levels similar to the control in RT, whereas the AI did not vary; c) the total number of Sc increased initially to reach values similar to the control d) the increase in Sc proliferation and the absence of variation in apoptosis led to the restoration of the Sc population during recrudescence.

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THREE-DIMENSIONAL ARCHITECTURE OF THE GOLGI COMPLEX OF EPIDIDYMAL PRINCIPAL CELLS

Narcisa Martínez-Martínez[1], Emma Martínez-Alonso[1], Mónica Tomás[2], José A. Martínez-Menárguez[1]

[1]Department of Cell Biology and Histology, Biomedical Research Institute of Murcia (IMIB Arrixaca-UMU), University of Murcia, 30100 Murcia, Spain
[2]Department of Human Anatomy and Embryology, Medical School, Valencia University,Valencia, Spain

Introduction: The principal cells from epididymis have one of the most developed Golgi complex (GC) known.
The unique environment of the epididymis allows the sperm to acquire motility and the ability to fertilize the oocyte. In the present study, we have used this cell as model for the study of thethree-dimensional architecture of the GC of highly secretory and endocytic cells.

Material and Methods: We have used electron tomography in combination with cryoimmunoelectron microscopy and classical transmission electron microscopy to study the organization of the GC in this cell type. More than forty 3D-reconstructions of the perinuclear cytoplasm were performed.

Results: Electron microscopy analyses of principal epididymal cell revealed the extraordinary complexityof the Golgi architecture, which appeared as a very large network composed of branching interconnected cords covering an extensive region of the supranuclear area of the cell. Electron tomography demonstrated the presence in this cell type of some unknown or very unusual Golgi structures such as branched cisternae, pocket-like cisternal invaginations or tubular connections. In addition, we found a close relationship between this organelle and both the endoplasmic reticulum (ER) and microtubules. ER cisternae surrounded cis and trans Golgi sides. At the cis side, ER membranes lied adjacent to both the cis-most Golgi cisterna and the discrete polymorphic membranous elements belonging to the ER-Golgi intermediate compartment (ERGIC). The extensive trans-ER formed close contacts with the two trans-most cisternae. In addition, we also observed ER-derived tubules emerging from ER inwards the trans cisternae. Microtubules were numerous in the Golgi region of principal cells and were also found traversing the Golgi ribbon at multiple points.

Conclusions: Despite of the huge numbers of studies, the GC is still giving surprises. This organelle may have unusual elements in specialized cells, the role of which remains to be clarified.

α7-NICOTINIC RECEPTOR EXPRESSION IS AT HIGHER DENSITIES AT THE EQUATORIAL BAND IN HUMAN SPERM CELLS

Mazen Fakih[1], Alba De Juan[1], Jose L. Girela[1], Maria M. Francou[1], Rafael Bernabeu[2], Joaquín De Juan[1]

[1]Departament of Biotechnology, University of Alicante, Alicante, Spain.
[2]Instituto Bernabeu of Reproductive Medicine, Alicante, Spain.

Introduction: Nicotinic Acetylcholine receptors (nAChRs) are ligand-gated cation channels involved in fast synaptic transmission in the central and peripheral nervous system. They form a heteropentameric structure with several combinations of α and β subunits, while the α7, α8, and α9 subunits may form homomeric receptors. There are nine different α subunits (α2 – α10) and three different β subunits (β2 – β4). All subunits have been described in mammalian tissues except for α8. There is much evidence to support that nAChRs are expressed on the membranes of mammalian sperms. In such a case, the presence of molecules belonging to the cholinergic system serve in the inter- and intra-cellular signaling mediated by downstream calcium channels. In this study, we aim to show the distribution of α7 nAChR molecules in human sperm cells using Confocal Laser Scanning Microscopy (CLSM) and Field Emission Scanning Electron Microscopy (FESEM).

Material and Methods: Sperm samples were obtained from healthy male adults by masturbation and were processed within less than an hour of the acquisition of the sample. A complete spermiogram was performed per the evaluation criteria of the World Health Organization (2010) for each of the sperm samples. Immunohistochemical reactions were performed on suspensions of sperm by the indirect immunofluorescence method. The primary antibody used is an Anti-Nicotinic Acetylcholine Receptor alpha 7 antibody (ab10096) manufactured by Abcam (Cambridge, United Kingdom). CLSM and FESEM were used. CLSM images were analyzed with the Carl Zeiss Zen 2.3 lite and FESEM with a ZEISS Merlin VP Compact.

Results: CLSM showed that the α7 nAChR are mostly distributed in the acrosomal area and the post-acrosomal area but are more concentrated in an equatorial band. FESEM imaging showed a high density of the receptors in the equatorial band area where they form small clusters. This specific spatial distribution of the α7 nAChR suggests that the acrosome reaction is initiated in the equatorial band area.

Conclusions: These data suggest that that the acrosome reaction is initiated at the equatorial region when the α7 nAChR acts as the trigger.

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ACROSOMAL FAILURE BIOGENESIS CAUSES GERM CELL NUCLEAR FACTOR (GCNF) OVEREXPRESSION IN GLOBOZOOSPERMIC GOPC-/- MICE

Maider Bizkarguenaga[1], Laura Gómez-Santos[1], Juan Francisco Madrid[2], Francisco José Sáez[1], Edurne Alonso[1].

[1]Department of Cell Biology and Histology, School of Medicine and Nursing, University of the Basque Country (UPV/EHU) Leioa, Spain.
[2]Department of Cell Biology and Histology, School of Medicine, University of Murcia, IMIB-Arrixaca, Murcia, Spain

Introduction: Abnormal acrosome formation results in a severe alteration of the sperm head called globozoospermia, a disorder of male sterility characterized by the presence of spermatozoa with round head and absence of acrosome. GOPC protein is involved in the processes of formation and transport of vesicles from the Golgi apparatus to the perinuclear region, where the preacrosomal vesicles merge. Gopc$^{-/-}$ mice have a defective vesicular traffic which causes an unusual accumulation of the pre-acrosomal vesicles, like in human globozoospermia. A role in the regulation of gene expression during gametogenesis in the adult has been suggested for GCNF. It would act as a transcriptional repressor of proteins (Oct4 among others). GCNF is localized in germ cells in both the testis and ovary; in heterochromatic regions of pachytene spermatocytes and round spermatid nuclei, and also in the spermatozoa acrosome.

Material and methods: Immunohistochemistry for optical and electron microscopy were performed with anti-GCNF on testis sections of wild type mice and Gopc$^{-/-}$ mice. For light microscope, testis were fixed in Bouin, embedded in paraffin and sectioned at 4µm. For immunogold electron microscopy, the samples were fixed in 2% glutaraldehyde, embedded in Lowicryl® K4M and sectioned at 70nm.

Results: In the wild type mice GCNF was moderately expressed in the nucleus throughout the spermatogenesis, reaching maximum values during the elongation of the spermatid. However, in the Gopc$^{-/-}$ nucleus two expression peaks were observed, the first one during the meiosis I and the second one around steps 13-16 spermatids. GCNF was also localized in the manchette in both mice type, with much higher expression in Gopc$^{-/-}$ spermatids.

Conclusions: The different expression of the GCNF protein in the Gopc$^{-/-}$ mice in the nucleus could suggest that the defective spermiogenesis deregulates the proper function of this protein in the gamete formation. In addition, its higher expression in the manchette of the round spermatids of Gopc$^{-/-}$ mice suggests that GOPC protein could be implicated in intra manchette traffic.

Acknowledgements: Immunoelectron micrographs were obtained in the Advanced Research Facilities (SGIker) at the Analytical and High-Resolution Microscopy in Biomedicine Service of the University of the Basque Country (UPV/EHU). This work was supported by grants from the UPV/EHU (EHUA13/15 and UFI 11/44).

GROUP-WORK PRACTICES TO INCREASE ACTIVE PARTICIPATION IN MEDICAL HISTOLOGY

Fernando Leiva-Cepas, Ignacio Ruz-Caracuel, Antonio J Agüera-Vega, Julio Osuna-Soto, María Jesús Gil-Belmonte, Ignacio Jimena, Evelio Luque, José Peña*

Department Morphological Sciences, Section of Histology, Faculty of Medicine and Nursing. University of Córdoba. Spain.

** Present address: Department of Pathology. La Paz University Hospital, IDIPAZ. Madrid.Spain.*

Introduction: Two original practical sessions have been developed for Histology teaching. Students have to face a problem and solve it in small groups using what they have learnt during previous classes and practices.

Methods: The first practical session consist of several histological images very similar in which students have to identify the distinguishing elements to make a differential diagnosis. After that, they have to reach a certain diagnosis in each image. In the second practice, students have histopathological images in which they have to identify the residual “normal histology” among the “distorted” elements, in order to identify the organ. Both practical session took place in a multimedia room, properly equipped with ICTs for small group sessions.

Results: Each group independently and using different resources, reach a diagnosis and complete a report. After both sessions, students were interviewed about its usefulness, complementarity and effectiveness to reach CE22, CT12 CT10 and CT 19 competences.

Conclusion: Results support this new approach in Histology practical sessions. They are well received by students and useful to put in practice necessary abilities in medical formation.

AUTONOMOUS USE OF THE VIRTUAL MICROSCOPE TO PREPARE THE LABORATORY PRACTICES

Iker Badiola, Francisco José Sáez.

Department of Cell Biology and Histology, School of Medicine and Nursing, The University of the Basque Country UPV/EHU, Spain

Introduction: The virtual microscopes available on the web provide us an additional tool to teach histology. Our students prepared the practical classes of human histology using virtual microscopes looking slides similar to those anticipated in each practice. At the end of the semester, we conducted a survey of our students to know their opinion and to know what their method of work was.

Material and Methods: The students indicated their degree of agreement (on a scale from 5, total agreement, to 0, total disagreement) with the following statements:

1. Observing the slides with the virtual microscope has made it easier for me to study them in the laboratory.
2. Preparing the practices with a virtual microscope is a waste of time.
3. Working with a virtual microscope is easy.
4. The slides of the virtual microscope should be the same as those of the laboratory.
5. It is difficult to find suitable slides for each practice in virtual microscopes.
6. Overall, I am satisfied that I have worked with the virtual microscope.

In addition, they were asked about the frequency with which they consulted histology atlas and about their working method.

Results: Analyzed the responses, the mean of according to the statements were: 4.48 for statement 1, 1.76 for statement 2, 2.36 for statement 3, 3.08 for statement 4, 3.32 for assertion 5 and 3.8 for assertion 6.
Two students always used histology atlases, eight used them many times, six sometimes, three rarely, and never six.
Finally, most of the students sought the slides in the virtual microscopes without the help of other colleagues, although many of them studied the objectives that the virtual microscope itself pointed out.

Conclusions: Students agree that the method used facilitates subsequent work in the microscopy laboratory and, although the work is not easy, they feel satisfied.
Not many of them used histology atlases to prepare their work.
Most of them have done an autonomous search of the slide, although later they moved by it with the indexes of the virtual microscope.

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COMPARISON OF THE LEARNING ENVIRONMENT IN THE HISTOLOGY PRACTICES OF FIVE UNIVERSITIES AND TWO DEGREES: MEDICINE AND BIOLOGY

Joaquín De Juan[1], Rosa M. Pérez-Cañaveras[2], Alba De Juan Pérez[1], José L. Girela López[1], José Peña Amaro[3], Manuel Garrosa[4], Juan F. Madrid[5], Rosa Noguera[6].

[1]Department of Biotechnology. Faculty of Science. University of Alicante, Alicante, Spain.
[2]Department of Nursing. Health Sciences. University of Alicante, Alicante, Spain.
[3]Department of Histology. Faculty of Medicine. University of Córdoba, Córdoba, Spain.
[4]Department of Cell Biology, Histology and Pharmacology. Faculty of Medicine. University of Valladolid, Valladolid, Spain.
[5]Department of Cell Biology and Histology. Faculty of Medicine. University of Murcia, Murcia, Spain.
[6]Department of Pathology. Faculty of Medicine. University of Valencia, Valencia, Spain

Introduction: Histology is a fundamental discipline for the training of biologists and physicians. By its microscopic nature, the practices are developed in laboratories equipped with microscopes. The implementation of laboratory practices in science teaching providing significant advantages to teachers and students. Among them, we have (1) integration of knowledge, tasks and resources of learning, (2) provide teachers with new skills and resources for their work and (3) enable students to integrate cognitive, emotional and sensory-motor domains into their laboratory tasks. Currently, the "learning environment" also is considered as a component of the teaching-learning process and very useful for assessing the quality of teaching of a particular subject. The aim of this paper is to gather the opinion of the Histology students of five Spanish universities (Alicante, Córdoba, Murcia, Valencia and Valladolid) and two different degrees: Medicine and Biology.

Methods: We used Fraser's "Science Laboratory Environment Inventory" (SLEI), abbreviated by Lightburn and translated and adapted to Spanish by De Juan et al. (1916) with the acronym of SASLEI. It consists of 24 questions grouped into four categories: integration of theoretical and practical contents, rules of operation in the laboratory, cohesion among students and quality of materials and infrastructures. Each question consists of five answers whose value oscillates between "almost never" and "very often". The questionnaire was applied to the corresponding groups of students of Histology, mentioned above. The data were analysed according to the previously published methodology (De Juan, et al., 2016).

Results: The students value the four SASLEI categories positively. Globally considered, of all of them the most valued is the cohesion category. The differences between universities, gender and degree, were established and discussed.

Conclusion: SASLEI is a comfortable and discriminating tool in the learning environment of Histology practices.

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AN INTERACTIVE ONLINE EVALUATION SYSTEM USING MOBILE PHONES AND COMPUTERS

Pedrosa J.A.[1], Peinado M.A.[1], del Moral M.L.[1], Hernández R.[1], Blanco S.[1], Siles E.[1], Lomas R.[2], Molina F.J.[2], Osuna M.C.[2], Carmona R.[3] y Rus A.[3]

1: Experimental Biology, University of Jaén
2: Health Sciences, University of Jaén;
3: Cell Biology, University of Granada.

Introduction: The design and management of the different evaluation systems in the University has benefited from the incorporation of the Information and Communication Technologies, which have progressively converted into the Interactive Teaching / Learning Systems. Within these systems, platforms and applications have been developed to implement the evaluation process, making it interactive and supported by the use of different devices. The present study proposes the implementation of an online evaluation system, using tests designed through an Internet platform, and capable of analyzing the results in real time.

Material and Methods: We have used the *Socrative* platform, which is free of charge and accessible directly through any computer, mobile phone or tablet, or by prior installation of the application. The application allows both generating questionnaires with different types of questions and saving them for later use. Through this system, the teacher can also propose a question to the students in the classroom and check the progress of learning and degree of attention. Once the test is finished, the application displays the results in real time, sending a complete report of the results to the teacher.

Results: This evaluation system is being applied to subjects related to Histology in the degrees in Biology, Physiotherapy and Biotechnology of the Universities of Jaén and Granada. The results available have shown an increase in the students´ performance with respect to previous courses. Likewise, since the correct performance of the tests by the students has a positive impact on the final record of the subjects, the students have shown greater motivation and interest in such subjects.

Conclusions: The *Socrative* platform facilitates the teaching-learning process, so its use could be recommended, both after finishing the evaluation of the results obtained in the different subjects involved in this study and upon request to the appropriate instances, to other subjects and degrees.

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AN EXPERIENCE OF TEAMWORK IN HUMAN HISTOLOGY WITH DENTISTRY STUDENTS

Francisco José Sáez.

Department of Cell Biology and Histology, School of Medicine and Nursing, The University of the Basque Country UPV/EHU, Spain

Introduction
A teamwork experience has been carried out with Dentistry students of Human Histology. At the end of the semester, a survey was conducted in which the students were asked what their method of work was and their opinion on this type of work. In addition, a reflection is made on the validity of this technique.

Material and Methods
The students carried out seven exercises in groups of three and through non-presential work. The exercises had the same structure as the individual exams that are carried out periodically in the classroom. At the end of the course an anonymous survey was conducted to know the working method used by the students and to know their opinion.

Results
Many students did the work individually with a final pooling. Most of the students affirmed that they studied only the part of the subject necessary to solve the exercises. In addition, the students indicated their degree of agreement (on a scale from 5, total agreement, to 0, total disagreement) with the following statements: (1) the teamwork has helped me to study and learn the subject, (2) I would have learned more if I had done the work individually, (3) working as a team is a waste of time, (4) the teamwork have allowed me to get used to working as a team, and (5) the score and revision made by the teacher has allowed me to improve my learning. Analyzed the responses, the mean of according to the statements were 4.6, 2.5, 1.1, 3.5, and 4.3, respectively.

Conclusions
In most cases, the initial objective, that is, to study the whole subject and then work together, was not achieved,
In general, the students consider that the work done has helped them to learn the subject and to learn to work as a team.
Opinions are divided equally between those who think they would have learned more by working individually and those who do not think so.
It is not clear if teamwork is a good tool for learning.

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HISTOLOGY, A TOOL FOR POPULARIZATION OF SCIENCE IN URUGUAYAN SCHOOLS: THE TRAVELLING SUITCASES PROJECT

Nibia Berois [1], Nelsa Botinelli [2], Daniel Bergara [2], Michel Hakas [2].

[1]Cell Biology Department. School of Sciences. Universidad de la República. Montevideo, Uruguay.
[2] Ciencia Viva Association. Montevideo, Uruguay.

I**ntroduction:** Ciencia Viva is a without-profit Civil Association belonging to the RedPop, UNESCO. Funded in 1993 was pioneer in Uruguay in the organization of interactive Science-Technology museums. The main objectives are to establish a dialogue among science and society, to stimulate the reflexion and to inquire about the science in the everyday life. Ciencia Viva offers a Museum in Montevideo, an inclusive place for children and adults, thematic travelling country exhibitions, workshops for teachers, etc. One project is “Travelling Suitcases”: Energy, Communication, Perception, Sensors-Measures and Life. The proposal was to bring scientific support to schools placed in towns with less than 7000 habitants. The project took advantage of the government program “One child-one computer” that provided free laptop to every child in Uruguay. The Life Suitcase explores different levels on the organization of living organisms.

Materials and Methods: The Life Suitcase contains: pendrive with instructions, links to a video that show levels of organization (from organism to molecules), videos and images about tissues and cell organization, microscope, tissues slides, glass slides to make fresh histological preparations, pipets and disposable material plus the chemical needed in the experiments. The methodology is totally interactive and the children have to made all the activities, from the examination of an organism (any living been they can bring) to the extraction of DNA from a fruit. The suitcase also has posters with instructions and worksheets to register the activities and the evaluations of the teachers.

Results and conclusions: After 2 years, we could verify that this interactive proposal has been well received. The teachers appreciate to count on with such materials in their school, so far of the cities. They also are excited about the interactivity and autonomy of the children making experiments and the close relationship between the scientific topics and the everyday life. We will show quantitative data about the Life Suitcase experience in all our country.

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NORMAL AND PATHOLOGICAL ANIMAL ANATOMY AND HISTOLOGY IN A LEARNING PRESENTIAL – VIRTUAL ENVIRONMENT.

García Lorenzana Mario[1] and Beltrán Vargas Nohra Elsy[2].

[1]Area de Neurociencias, Departamento de Biología de la Reproducción. Universidad Autónoma Metropolitana Iztapalapa. Av. San Rafael Atlixco 186, Col. Vicentina. Ciudad de México, 09340, México.
[2]Departamento de Procesos y Tecnología, Universidad Autónoma Metropolitana – Cuajimalpa. Av. Vasco de Quiroga 4871, Col. Santa Fe. Ciudad de México. 05300, México

Introduction: The educational model at the Autonomous Metropolitan University-Iztapalapa (UAMI) is a classroom course. However, teachers are permanently incorporated into virtuami, an institutional resource that allows distance education by generating virtual classrooms (VC) to support the units of teaching learning (UUEEAA) of different divisions that makes the academic structure of the UAM. In particular, in the Biological and Health Sciences Division, we participated in the creation of a virtual classroom for UUEEAA Histology and Animal Anatomy (HAA), and Animal Histopathology (AHP). In this sense, it was very important to develop pedagogical mediations as well as appropriate didactic approaches, for a model in which the presence is supported by virtuality. To communicate the UAM experience in the development of the virtual learning environment for UUEEAA HAA and AHP.

Material and Methods: The UEAEAA HAA and AHP programs are designed per our own didactic approach (combining a constructivist and competencies approach). These didactic proposals are based on a disciplinary approach (theoretical, methodological and technical), which seeks to improve the teaching task through a strategy that considers: 1) socio-economic factors; 2) the students time and the institutional time; 3) material and human resources; 4) specialized language; 5) activities and didactic material, and 6) the reasoning forms proper to the knowledge of the discipline.

Results: As part of the (UUEEAA) program, a bibliographical and hemmerographic anthology, an internship manual, and a computer resource for support (material available in VC) were developed. The evaluation is permanent and ponders the congruence between objectives of the UUEEAA program and the educational strategy proposed. The virtual classroom is seven years old, equivalent to 21 trimesters in which UUEEAA HAA has been taught. At the end of this time we have been able to discern the strengths and weaknesses of this learning environment.

Conclusions: By contrasting the grades between the courses that are only onsite and face-to-face with virtual supports, the latter shows a better performance.

IDENTIFICATION OF INDUCIBLE NITRIC OXIDE SYNTHASE AS DAMAGE TISSUE MARKER GENERATED BY ISCHEMIA-REPERFUSION IN GASTRIC MUCOSA

*Peña-Mercado, Eduardo [1], *García-Lorenzana, Mario [2], Aréchaga-Ocampo, Elena [3] González- de la Rosa, Claudia H.[3] y *Beltrán-Vargas, Nohra E [4]*

[1] Posgrado en Ciencias Naturales e Ingeniería. Universidad Autónoma Metropolitana-Cuajimalpa.
[2] Departamento de Biología de la Reproducción, Universidad Autónoma Metropolitana – Iztapalapa. Av. San Rafael Atlixco 186, Col. Vicentina. Ciudad de Mexico, 09340, Mexico.
[3] Departamento de Ciencias Naturales, Universidad Autónoma Metropolitana – Cuajimalpa
[4] Departamento de Procesos y Tecnología, Universidad Autónoma Metropolitana – Cuajimalpa. Av. Vasco de Quiroga 4871, Col. Santa Fe. Ciudad de Mexico. 05300, Mexico.

Introduction: Hypovolemic shock is a pathological condition in which there is a global insufficiency of tissue perfusion leading to inadequate delivery of oxygen and nutrients to meet the needs of the tissues and it is one of the main causes of mortality in critically ill patients. Gastric ischemia-reperfusion damage is a clinical problem associated with the course of hypovolemic shock. Inducible nitric oxide synthase (iNOS) expression is related to ischemia conditions and the production of nitric oxide from it, generates damage to the gastric mucosa. Impedance spectroscopy has been used to study the tissue damage generated by ischemia. The aim of this work was to identify and relate iNOS expression to tissue damage and impedance variations generated by I/R in gastric mucosa.

Materials and Methods: Male Wistar rats (350-450 g) were used for the study. All procedures were performed according to NOM-062-ZOO-1999. Three groups were studied: control, ischemia and I/R for 60 min. Ischemia was produced by celiac artery clamping for 30 min, followed by a reperfusion period of 60 min. Impedance spectra and biopsies were taken for histological and immunohistochemical analysis for iNOS detection.

Results: In the control group, gastric mucosa was observed in normal conditions. The ischemia group presented characteristic histological changes of acute inflammatory process. In the I/R group, epithelial erosion and necrotic process cells were identified. The quantitative analysis of tissue damage presented a statistically significant increase ($p < 0.01$) in the I/R group with respect to the control and ischemia groups. Immunohistochemical analysis to identify iNOS expression presented a statistically increase ($p < 0.01$) in the number of immunoreactive cells in the I/R group with respect to the control and ischemia groups. Gastric impedance showed a statistically significant increase ($p < 0.05$) in the I/R group indicating greater damage than in the ischemia group.

Conclusions: iNOS expression can be related to tissue damage and gastric impedance variations, and could be a marker of tissue damage in I/R conditions in the gastric mucosa.

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POSTER SESSIONS

USE OF VIRTUAL MICROSCOPY IN A BLENDED LEARNING MASTER DEGREE

Eva Martinez-Pinilla[1], Eva del Valle-Suárez[1], Jorge Tolivia-Fernández[1], Ana Navarro-Incio[1]

[1]Department de Morphology and Cell Biology, Faculty of Medicine and Health Sciences, University of Oviedo, Spain

Introduction: The Master Degree in Neurosciences Research of the University of Oviedo is a blended learning mode one. In the case of our optional subject "Recent studies in aging and neurodegeneration" the face-to-face classes are only the 30% of 3 ACTS credits consequently, access to microscopes and histological slides is limited to a few hours and not enough for the adequate study of the practical content of the subject. Therefore, we present a proposal for the use of virtual images of histological preparations. The specific aims of this project were: creation of a data base of digital images (virtual repository of specimens) that allows the computer simulation of a photonic microscope with free software (Virtual Microscope) and testing of student final satisfaction.

Material and Methods: Virtual repository was created with a high resolution Leica scanner and consisted in several nervous system tissue samples from both control and aged subjects, with or without neurodegenerative diseases, prepared with different histochemical and immunohistochemical techniques. The key features of the images in the repository are explained by the teachers during the face-to-face sessions and the students could observed them using a photonic microscope in the lab that later they could review at home using a Leica software. In order to evaluate the quality and usefulness of the designed tool, a satisfaction survey was passed to the students.

Results: Only 20% of the students had never used any type of microscopy. These students, curiously, preferred the use of the real microscope over the virtual one. The rest of the students preferred to use both types of microscopes for the practical study of the histological preparations. The majority of students did not find difficult to use the program, but to install and load these too large images.

Conclusion: The students prefer and see more effective the combined use of the two types of microscopes (real and virtual) in the practical teaching of this subject.

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DARK NEURONS, UNWANTED GUESTS IN SOME FIXATION METHODS

Ana Navarro-Incio[1 2],Marina Parrondo-Costales[1], Eva del Valle-Suárez[1 2], Eva Martinez-Pinilla[12], Jorge Tolivia-Fernández[1 2]

[1]Department de Morphology and Cell Biology, Faculty of Medicine and Health Sciences, University of Oviedo, Spain

[2] Instituto de Neurociencias de Principado de Asturias (INEUROPA), Spain

Introduction: Brains of experimental animals for histological or immunohistochemical use are usually obtained after intracardiac perfusion with different fixatives. The brain is then removed from the skull and postfixed for about 12 to 18 hours. The fixatives used are varied but the current world trend for immunohistochemical studies is to fix with 4% paraformaldehyde in phosphate buffer or in Bouin's solution. The systematic appearance of certain neurons in apparent degeneration in the brains of young, healthy control animals after fixation with paraformaldehyde led to the search of possible reasons for this phenomenon.

Material and methods: These neurons were studied using various histochemical and immunohistochemical techniques and under different fixation conditions for light and electronic microscopy.

Results: Dark neurons appeared only when they were fixed exclusively with aldehydes and never when we used them in combination with other fixative agents. These cells often appeared as a degenerated large neurons located in isolation among populations of apparently healthy neurons, usually in cortical regions such as the anterior cingulated area, the motor area, the somatosensory area, the piriform area and the olfactory tubercle. Light microscopy showed that they presented cytoplasmic hyperbasophilia, nuclear pyknosis, apical dendrites with irregular appearance and massive contraction of the whole cell, among other characteristics. Electron microscopy showed that such neurons were shrinked and much more electrondense than the others. However, they did not exhibit any of the characteristics associated with a cell in degeneration and/or cell death. In addition, these cells appeared surrounded by dilated and edematous neurites.

Conclusions: Since dark neurons appeared only in brains fixed with paraformaldehyde, but not in those fixed with Bouin's solution it seems that the composition of the fixative could be responsible for these artefacts. On the other hand, dark neurons are preferentially found in those areas under instrumentally pressure when the brain is extracted, so it is reasonable to think that dark neurons presence is due to a combination of mechanical and chemical events. To avoid the risk of misinterpretation of the results in the experiments, the fixative used should be well chosen and the fixation protocol accurately controlled.

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PI3K PATHWAY IN PROSTATE CANCER

Benito Fraile[1], Norelia Torrealba[1], Gabriel Olmedilla[2], Pilar Martínez-Onsurbe[2], Raúl Vera[1], Ricardo Paniagua[1], Mar Royuela[1].

[1]Department of Biomedicine and Biotechnology, University of Alcalá. Madrid, Spain
[2]Department of Pathology; Príncipe de Asturias Hospital. Alcalá de Henares. Madrid, Spain

Introduction: The PI3K/AKT/mTOR pathway plays an essential role in a wide range of biological functions in normal cells, regulating in a cell - and stimulus - dependent manner processes such as proliferation, cell cycle, DNA repair, differentiation, survival, apoptosis, senescence and metabolism. Consequently, deregulation of this pathway often leads to complications, such as cancer. This study focuses on PI3K/AKT/mTOR pathway as the consequences of this deregulation in the development of prostate cancer (PC).

Material and Methods: The expression of components of the PI3K/AKT/mTOR pathway was analysed by immunohistochemistry in transurethral resections or radical prostatectomies from 86 men (aged from 52 to 74 years) with prostate cancer and histologically normal prostates (NP) from 10 men (aged from 20 to 38 years) without histories of reproductive, endocrine or related diseases.

Results: In normal prostate, positive immunoreactions were found in the secretory compartment exclusively for pAkt-Ser and pAKT-Thr, while all the proteins except PI3K were detected in the stromal compartment. In prostate cancer samples, a perinuclear immunoreaction pattern was found for all proteins. The optical density analysis revealed an increased expression in PC compared to NP for all the proteins apart from mTOR (epithelium and stroma).

Conclusions: The PI3-K/Akt/mTor pathway is known for its frequent activation and deregulation in tumorigenesis due to amplification and mutations in the gene encoding for PI3K and other family members. Constitutively activate PI3-K/Akt/mTor promotes cellular survival and resistance to chemotherapy and radiation in prostate cancer cells. Our results suggest a role for prostatic expression of PI3K as a prognostic marker for prostate cancer. The PI3K/AKT/mTOR pathway is becoming important therapeutic targets and biomarkers of the onset and progression of prostate cancer.

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ROLE OF ERK IN THE TREATMENT OF PROSTATE CANCER

Benito Fraile[1], Norelia Torrealba[1], Gabriel Olmedilla[2], Pilar Martínez-Onsurbe[2], Raúl Vera[1], Ricardo Paniagua[1], Mar Royuela[1].

[1]Department of Biomedicine and Biotechnology, University of Alcalá. Madrid, Spain
[2]Department of Pathology; Príncipe de Asturias Hospital. Alcalá de Henares. Madrid, Spain

Introduction: ERK is accepted to play a prominent role in cell survival and tumour promotion in response to a broad range of stimuli, including cytokines. However, there is a growing body of evidence supporting that ERK contribute to the development of a number of malignancies. The aim of the present work was to determine the effect on TGF-B that triggers the ERK inhibitor in prostate cancer cell lines dependent (LNCaP) and independent (PC3) of androgens, as well as the prostatic epithelial cell line without prostatic pathology (RWPE-1).

Material and Methods: LNCaP, PC3 and RWPE-1 cells were pre-treated with MEK (the upstream kinase of ERK) inhibitor (PD98059) and then exposed to TGF-B. Proliferation was quantified through MTT and flow cytometry. All data obtained with the flow cytometry technique were treated and analyzed using the Cyflogic v. 1.2.1 (CuFlo Ltd, Turki, Finland).

Results: It is observed that in the PC3 and LNCaP lines, in the presence of TGF-B, there are significant increases in cellular ratio in S / G2-M that coincides with the decrease in the same cell proportions in G0 / G1. The effect of the inhibitor alone does not show significant changes with the cellular populations in G0 / G1 whereas the percentage of cells in S / G2-M decreases slightly. When the inhibitor is combined with the cytokine, the proliferative effect of the TGF-B observed is not recovered

Conclusion: The inhibition of ERK in our cell lines yielded significant results. This leads us to conclude that the ERK inhibitor should be taken into account in the design of future treatments for prostate cancer. This is confirmed by the observation of the number of metabolic pathways that converge in the activation of ERK.

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ICTIOHEMATOLOGYCAL BIOMARKER AS A TOOL IN THE MONITORING OF AQUATIC CONTAMINATION

Brian Real Huescas[1], Misael Hernández Diaz[1], Irma Hernández Calderas[1], J. Roberto Jerónimo Juárez[1], Xochitl Guzmán-García[1].

[1]Departamento de Hidrobiología. División de Ciencias Biológicas y de la Salud. Laboratorio de Ecotoxicología, Histopatología. Universidad Autónoma Metropolitana Unidad Iztapalapa. Ciudad De México, México.

Introduction: In ecotoxicology; the application and validation of biomarkers is fundamental, due to the information that is generated from the effects produced by toxic substances. The study of fish blood as biomarker of aquatic pollution, results in high impact, considering the tissue as means of transport of toxics to different organs, this may conduce alterations in the organism. Ictiohematologycal analysis becomes key to provide the diagnosis and control of diseases, as well as the ecological impact associated with the reference zone. The aim of this study was to characterize the different groups of blood cells and morphological changes in nuclear and cytoplasmic cells of fish belonging to the localities of Tecolutla, Veracruz, Manantlan Jalisco and Valle de Bravo, Estado de México.

Material and methods: Organisms were collected and anesthetized with 5% benzocaine in the mentioned localities. Blood by cardiac puncture was extracted and blood smear were made, allowing them to air and submerged in absolute alcohol for its fixation. Subsequently, they were stained with Giemsa dye and analyzed in an optical microscope with a 100x.

Results: Different types of cells were observed, such as: basophils, eosinophils, lymphocytes, monocytes and some blast cells; observing greater morphological alteration (poikilocytosis) in erythrocytes, such as: erythroblasts, equinocytes, lysed erythrocytes and Heinz bodies. In abnormalities associated with hemoglobin (anisochromia) was observed: hyperchromia, hypochromy and polychromia. From the diversity of cells that prevailed in the samples and from the alterations analyzed, it could be indicated that fish health is more affected in Tecolutla Veracruz and Manantlan, Jalisco.

Conclusions: It was possible to characterize the cells and hematological alterations in the studied fish, noting that the organisms with the highest prevalence of alterations were Tecolutla Veracruz and Manantlan, Jalisco. The use of ictiohematologycal biomarkers is recommended for the early detection of environmental hazards, as well as their consequences on aquatic organisms.

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LEAF HISTOLOGY OF GENUS *PINUS* FROM DURANGO STATE

Carmen de la Paz Pérez Olvera[1]*, Jacqueline Ceja Romero*[1]*.*

[1]*Department of Biology, Universidad Autónoma Metropolitana unidad Iztapalapa. Mexico City.*

Introduction: Mexico is the country with the largest number of *Pinus* species with about 111, in Durango are 20 registered. In the country the information is scarce about the histology of the leaf of this genus, however the knowledge of some histological characters are applicable in taxa classification and identification. The pines are evergreen trees, with acicular leaves arranged in fascicles, in varying numbers and protected at the base by a persistent or deciduous sheath. The goal of this work was described the histology of the leaf of *Pinus arizonica*, *P. cooperi*, *P. durangensis*, *P. herrerae*, *P. leiophylla* and *P. teocote*, collected in San Dimas and Santiago Papasquiaro, municipalities of Durango state, Mexico.

Material and Methods: Herborized material of each species was softened with OT aerosol. Samples of about 5 mm, were dehydrated with tert-butyl alcohol in concentrations of 30, 50, 70, 85, 96, 100% and then paraffin embedded technique was used. With a rotary microtome were obtained transversal sections of 25 µm, which were dewaxed and stained with astra blue-basic fuchsin protocol.

Results: In cross section the leaves of the six species have triangular shape with angles of different size. The epidermis is a single row of cells with thick walls, the stomata are sunken. The hypodermis is uni or multi-stratified. The mesophyll is homogeneous with undulated cells. The resin ducts were variable in number and position in the mesophyll and had different number of epithelial cells. The vascular system is formed by two central vascular bundles, surrounded by the transfusion tissue composed of tracheids and parenchyma.

Conclusions: The shape of the leaf is similar in all species, nevertheless the opening of the angles differs in some of them. Also the number and position of resin ducts vary according to taxa, therefore these characters have been considered useful in the identification of species

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TRANSCRIPTION FACTOR: CORRELATION WITH CLINICOPATHOLOGICAL FEATURES AND PROGNOSTIC VALUE IN PROSTATE CANCER

Concepción Chaves[1], Benito Fraile[1], Norelia Torrealba[1], Gabriel Olmedilla[2], Pilar Martínez-Onsurbe[2], Raúl Vera[1], Ricardo Paniagua[1], Mar Royuela[1].

[1]Department of Biomedicine and Biotechnology, University of Alcalá. Madrid
[2]Department of Pathology; Príncipe de Asturias Hospital. Alcalá de Henares. Madrid, Spain

Introduction: Prostate cancer is one of the most extended malignant. It is characterized by a multifocal, slow-growing. The Prostate-Specific Antigen (PSA) test is used in its diagnosis years ago, although with limitations. Because of these, other possible factors are researched in order to help the prostate cancer diagnosis. The aim of the present work was to determine the influence of transcription factors (NF-kB, Elk-1, ATF-2, Ap-1) on the biochemical progression of prostate cancer.

Material and Methods: To evaluate the association between clinic pathological and immunohistochemical variables Spearman's test was performed. Log-rank test and Kaplan- Meier curves were used for survival comparisons. To explore the correlation of the studied immunohistochemical parameters with biochemical progression, univariate and multivariate Cox proportional hazard regression analyses were performed.

Results: Spearman analysis showed a correlation between stromal expression of p-ATF-2 with preoperative serum PSA levels; and tumour expression with perineural invasion. A correlation was also found between stromal expression of AP-1 with pathological T stage; and tumor with pathological T stage. A correlation was also found between tumor expression of p-Elk-1 with Gleason score and survival. Inversely a correlation was found between stromal expression of p50 and pathological T stage, perineural invasion, margin status and survival. In the multivariate Cox regression model none of these transcription factors can be considered as predictive of the disease.

Conclusions: After the correlation of Immunohistochemistry data with classic markers, it was determined that none of these transcription factors can be considered as predictive of the disease. These transcription factors are activated as a consequence of the activation of different transduction pathways. Therefore, their action is secondary to the development of the disease.

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XILOGLUCAN, HIBISCUS AND PROPOLIS AS A BARRIER AGAINST URINARY INFECTIONS

Concepción Chaves[1], Javier Alcover[2], Mar Royuela[1], David Rodríguez[2], Ricardo Palacios[2], Benito Fraile[1]

[1]Department of Biomedicine and Biotechnology, University of Alcalá. Madrid
[2]DIATER Laboratories. Avenida Gregorio Peces Barba, nº 2. Parque Tecnológico de Leganés.

Introduction: The medical device containing xyloglucan, hibiscus and propolis (Noventure Spain) is a non-pharmacological oral supplement that was approved recently for the prevention of urinary tract infections. The aim of this work is to assess the properties of a medical device containing xyloglucan, propolis and hibiscus to create a bio-protective barrier to avoid the contact of urophatogenic E. coli (UPEC) strains on cell walls in models of intestinal (CacoGoblet) and uroepithelial (RWPE-1) cells.

Material and Methods: Two UPEC strains (expressing type 1 fimbriae and P fimbriae) were used to assess by electronic microscopy and ELISA the barrier properties of the medical device. The antimicrobial activity was assessed in broth dilution assays.

Results: The three components (xyloglucan, propolis and hibiscus) did not alter E. coli cell integrity in intestinal and uroepithelial cell models and were devoid of antibacterial activity. The three components avoided bacterial contact in both cell monolayers.

Conclusions: The non-pharmacological barrier properties of xyloglucan, propolis and hibiscus confirm the role of the medical device for the management of UTIs.

Acknowledgements: The study was supported financially by Novintethical Pharma, SA, Switzerland.

SEX-DEPENDENT CO-OCCURRENCE OF HYPOXIA AND B-AMYLOID PLAQUES IN HIPPOCAMPUS AND ENTORHINAL CORTEX IS REVERSED BY LONG-TERM TREATMENT WITH UBIQUINOL AND ASCORBIC ACID IN THE 3XTG-AD MOUSE MODEL OF ALZHEIMER'S DISEASE

Javier Frontiñan-Rubio[1], Francisco J. Sancho-Bielsa[1], Juan R. Peinado[1], Darío Díaz[2], Lydia Giménez-Llort[3], Mario Durán-Prado[1] and Francisco J. Alcain[1]

[1]Departmento de Ciencias Médicas, Facultad de Medicina de Ciudad Real, Universidad de Castilla-la Mancha .[2]Departamento de Psicología, Facultad de Medicina, Universidad de Castilla-la Mancha. [3]Departamento de Psiquiatría y Medicina Forense, Facultad de Medicina, Universidad Autonoma de Barcelona.

Introduction*:* Diverse structural and functional abnormalities in the cerebral microvasculature have been described both in patients and animal models of Alzheimer disease (AD). One causing hypoperfusion is the thickening of the cerebrovascular basement membrane due to a greater collagen-IV deposition around capillaries. Our work describes how these and other alterations in the cerebrovascular system in 3xTg-AD mice at advanced stages of AD (12-month-old) can be prevented by long-term diet supplementation with ubiquinol (Ub+ASC) stabilized with the Kaneka QH P30 powder containing ascorbic acid (ASC).

Materials and Methods: 3xTg-AD mice were treated from prodromal stages of disease (3 months of age) with Ub+ASC, the excipient, ASC, or a standard diet and compared to wildtype mice. Coenzymes Q9 and Q10 contained in plasma were determined by HPLC. Inmunofluorescence images where acquired with a Nikon Eclipse TiU inverted scope. The thickness of basement membrane in the cerebral microvessels (CVBMs) was determined in 10 small blood vessels per animal located in the hippocampal fissure. Aβ deposition, hypoxia and BACE1 levels were determined on CA1 and entorhinal cortex analysing 2 slices per animal. The number of microglia was carried out in hippocampus CA1 in areas with absence of amyloid plaques analysing 2 slices per animal.

Results: Sex-related differences were found, as increased number of amyloid plaques in the hippocampus and entorhinal cortex of females. Only the areas in females with extensive β-amyloid peptide (Aβ) burden showed large hypoxic regions that colocalized with the Aβ plaques. Such neuropathological status could be reversed by Ub+ASC and, to a lesser extent, by ASC. Independently of the Aβ burden, increased collagen-IV deposition in the cerebrovascular basement membrane was found in both males and females 3xTg-AD mice, and was strongly reduced in Ub+ASC- and ASC- treated animals as compared to wildtype levels. Hippocampal chronic inflammation and peripheral leukocytes-oxidative stress found in 3xTg-AD mice were reversed by antioxidants.

Conclusions: The results of the current study define the antioxidants ubiquinol and ascorbate like key mediators in the cerebrovascular response to hypoxia and Aβ deposition and make them attractive molecules to prevent AD associated thickness during early asymptomatic stages of the disease.

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EFFECT OF UVB RADIATION IN THE EXPRESSION OF PROTEINS RELATED TO APOPTOSIS IN FRESHWATER PRAWN EMBRYOS

Heloísa Schramm[1], Eliane Zeni[1], Thaline Quadros[1], Michael L. Jaramillo[1], Yara Maria Rauh Müller[1], Dib Ammar[1,2], Evelise Maria Nazari[1].

[1]Departamento de Biologia Celular, Embriologia e Genética, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil.
[2]Centro Universitário Católica de Santa Catarina, Joinville, Santa Catarina, Brazil.

Introduction: In previous studies, we demonstrated that embryos of the freshwater prawn *Macrobrachium olfersii* exposed to ultraviolet B (UVB) radiation exhibited DNA damage, excessive ROS production, mitochondrial dysfunction and increased hsp70 expression, which are able, independently or together, to induce apoptosis. Then here, we studied the impact of UVB exposure on the expression of some apoptosis-related proteins (ARPs), such as p53, Bcl2, Bak, and Caspase3 in embryonic cells of *M. olfersii*.

Material and Methods: *M. olfersii* prawns were collected on Santa Catarina Island, Brazil (approved IBAMA 15294-1/2008), maintained in 60L aquaria, at 24 ±1°C, in a 12h light: 12h dark regime, fed once a day. Breeding occurred in aquaria and ovigerous females with embryos on the seventh embryonic day (E7) were irradiated with UVB lamp (dose 558J.cm^2) and embryos were analyzed in three time-points (3h, 6h, 12h) after exposure. Embryos were fixed in PEM-FA, embedded in tissue freezing medium and sectioned at 6 µm (ca. 100 embryos/time-points in triplicate). For TUNEL assay the TdTFragEL kit was used. The numerical density per area (NA) of TUNEL-positive cells was determined using the M-42 test system. For immunohistochemistry, sections were incubated with anti-p53, anti-Bcl2, anti-Bak, and anti-Caspase3 (1:100). For the analysis by flow cytometry, the embryonic cells in suspension were incubated with the same antibodies (1:1000) used for immunohistochemistry and the analyses were conducted using a FACSCanto II flow cytometer.

Results: We found an increase in the expression of p53 and Bak proteins just after 3h of exposure. After 12h, an increase in Caspase3 was observed concomitantly with an increased number of apoptotic cells. The expression of Bcl2 did not change in comparison to non-irradiated embryos.

Conclusions: Our data reveal that ARPs are modulated and chronological regulated in embryonic cells of *M. olfersii* and that UVB radiation causes apoptosis after 12h of exposure. Overall, we demonstrate that embryonic cells of *M. olfersii* are able to active the cell machinery against environmental changes, such as increased incidence of UVB radiation in aquatic ecosystems.

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HISTOPATHOLOGICAL CHANGES IN AORTA OF SPONTANEOUSLY HYPERTENSIVE RATAS LINKED TO ALTERATIONS IN MITOCHONDRIA AND CAPACITATIVE CALCIUM ENTRY

Iago Méndez López[1,2], Guilherme Henrique Souza Bomfin[1,3], Javier Regadera[4], Antonio G. García,[1,2], Fernando J. Padín[1,2,5].

[1]Instituto Teófilo Hernando de I+D del Medicamento, Madrid, Spain.
[2]Department of Pharmacology and Therapeutics, Faculty of Medicine, Autonomous University of Madrid, Madrid, Spain.
[3]Department of Pharmacology, University Federal of São Paulo (UNIFESP), São Paulo, Brazil.
[4]Department of Anatomy, Histology and Neuroscience, Faculty of Medicine, Autonomous University of Madrid, Madrid, Spain
[5]Department of Medical Sciences (Pharmacology), Faculty of Medicine, University of Castilla La Mancha (UCLM), Ciudad Real, Spain

Introduction: It is well known the vascular remodeling occurring during arterial hypertension, which are associated to functional changes in vascular smooth muscle cells (VSMC). To elucidate its origin, we have studied a cellular calcium influx process called capacitative calcium entry (CCE) and its relationship with the mitochondrial functionality.

Material and Methods: Immunofluorescence experiments to characterize and quantify of stromal interaction molecule 1 (STIM1) and calcium release-activated calcium channel protein 1 (ORAI1) protein expression were performed in both aortic tissue and cultured VSMC. Orcein and picrosirius red stained were used to analyzed the histomorphological differences in the thoracic aortic wall from WKY and SHR. CCE was analyzed with Fura 2-am dye in VSMC to measure the cellular calcium dynamic. The mitochondrial membrane potential was studied with tetramethylrhodamine ethyl ester (TMRE) and rhodamine (rhod2-am) fluorescent probes and their ultrastructure was analyzed by electron transmission microscopy.

Results: With respect to control WKY rats, SHR presented: i) higher capacitative calcium entry; ii) hypertrophic aortic wall remodeling; iii) higher expression of STIM1 protein but not ORAI1 in the aortic tissue and in VSMC; iv) lower mitochondrial membrane potential; v) mitochondrial ultrastructure disruption.

Conclusions: The results suggest that partially depolarized mitochondria could be the origin of the calcium dyshomeostasis occurring through an altered capacitative calcium entry. We propose a link between mitochondria and STIM1 protein expression that may aid drug development and therapeutic strategies to treat hypertension.

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CRYOPRESERVATION MODIFIES THE HUMAN SPERM MORPHOMETRIC CHARACTERISTICS WHEN OBSERVED WITH ATOMIC FORCE MICROSCOPE

Miguel Herreros[2], Alba De Juan[1], José Luis Girela[1], Jorge Ten[2], Rafael Bernabeu[2] and Joaquín De Juan[1]

[1]Department of Biotechnology. Faculty of Science. University of Alicante, Alicante, Spain.
[2]Instituto Bernabeu of Reproductive Medicine, Alicante, Spain.

Introduction. Cryopreservation implicates the freezing of living cells and tissues and its storage at -196°C or bottom. At this temperature, metabolic activity and lethal cellular changes are stopped. Sperm cryopreservation preserves the male fertility and its storage in a bank for future use. This technique is useful in several situations: (1) donor spermatozoa, from a bank of frozen samples, (2) Assisted Reproductive Technology, (3) non-traditional families, such as single women and homosexual couples, and (4) patients with medical and surgical interventions that could induce infertility, like cancer. The aim of this work is to apply the use of the Atomic Force Microscope (AFM) methodology for examining the morphological and morphometric changes of the sperm head following the process of freeze-thawing.

Material and methods. Semen samples were obtained from 4 healthy and normozoospermic young donors. The half of each sample were freezing and later defrosted for the study. Samples were processed according to WHO (2010). AFM analysis was carried out on spermatozoa in air medium using the NT-MDT equipment (Moscow, Russia). The images were acquired in the semi-contact AFM mode. The measurements were realised on the spermatozoa head. Eight shape parameters were evaluated: length, width, perimeter, area, height (maximum and minimum), roughness and roundness factor. A one-way ANOVA is performed by comparing the measurements obtained in fresh spermatozoa versus cryopreserved ones.

Results. When comparing the morphometric characteristics of the fresh sperm heads, with their homologous defrosted spermatozoa, are observed significant differences ($p < 0.1$) in some parameters. The action of freezing-thawing produced a significant decrease in head size except in the roundness factor and the maximum and minimum head heights. A significant roughness index increase is observed, probably because the cell membrane suffers a ruffled as consequence of decreasing of the head size.

Conclusions. Cryopreservation decreases the size of the heads of human spermatozoa and increases the cell membrane roughness. An atomic force microscopy is a useful tool to analysed with detail the morphology changes of sperm cells.

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ANALYSIS OF THE IMMUNOMODULATORY EFFECT OF DIETHYLCARBAMAZINE (DEC), ON NEW MECHANISMS OF ANTIINFECTIOUS RESPONSE IN HUMAN POLYMORPHONUCLEAR CELLS, (PILOT STUDY).

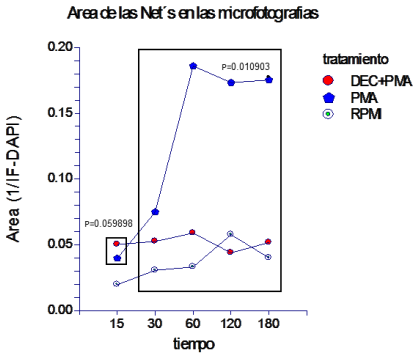
Juan Carlos Segoviano Ramírez[1,3], Andrés García Ramírez[3], Sandra de la Rosa Taméz[3], Jaime García Juárez[3], Mª de los Ángeles Castro Corona[2,3], Carlos Eduardo Medina de la Garza[2,3].

[1] UANL, Centro de Investigación y Desarrollo en Ciencias de la Salud (CIDICS), Unidad de Bioimagen.
[2] UANL, Centro de Investigación y Desarrollo en Ciencias de la Salud (CIDICS), Unidad de inmunomoduladores.
[3]UANL, Facultad de Medicina.

Introduction: Polymorphonuclear cells (PMN) play a key role in sites of acute infection where they destroy bacteria by oxidative and non-oxidative mechanisms. Neutrophil Extracellular Traps (NETs) are a newly described mechanism of innate response by PMN, consisting in complexes of DNA, histones, cytoplasmic granules that adhere and kill bacteria. Upon activation, PMN undergo nuclear fragmentation, envelope disorganizationwith exit of chromatin to cytoplasm and final expulsion to forma fine networks. NETosis is the death of PMN leading to NETs formation. PMN of diabetic people show an enhanced NETosis: delay in wound healing and diabetic ulcers is attributed to NETs. Piperazinderivate Diethylcarbamazine (DEC) has shown a modulatory effect on oxidative mechanisms in both macrophages and PMN by enhancing respiratory burst and cellular innate immune-response in granulomatous inflammatory processes. We tested the effect of DEC over NETosis in PMN from healthy young people.

Material and methods: Human PMN were incubated with DEC and later activated with NETosis inducer *Phorbol 12-myristate 13-acetate* (PMA). In a randomized double-blind model, NETosis was documented by confocal microscopy. Over the images, NETs were delimited and the fluorescence intensity (IF) of chromatin (stained with DAPI) and neutrophil elastase (with specific antibodies conjugated to Alexa fluor-488) was determined. NETs´ area was calculated at 15, 30, 60, 120 and 180 minutes.

Results: PMN of positive control group exhibited nuclear changes at 15' and started formation of NETs by 30'. In contrast, although PMN of the experimental group formed NETs, they appeared after 60' and were less numerous than those of positive control group at all times analyzed. Negative and internal control groups did not form NETs. The statistical testing of the NETs' area, by time and treatment groups, is shown in the graph. Quantitatively analyzed, formation of NETs in positive control group starts at 30', increases abruptly at 60' and then remains constant the rest of time. In experimental group, values were much lower (P = 0.010903 *) and this trend was maintained all over the experiment.



Conclusions: DEC decreases the capacity of healthy PMN to form NETs.

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EFFECT OF ULTRAVIOLET B IRRADIATION IN ROS LEVELS, SENESCENCE AND MIGRATION CAPACITY OF HUMAN FIBROBLASTS AND ADIPOSE DERIVED MESENCHYMAL STEM CELLS. A COMPARATIVE STUDY

Burón Aizpiri, M.[(1)], Palomares Casado, T.[(2)], Garrido-Pascual, P.[(1)], Zubillaga Marañón, V.[(1)], Alonso-Varona, A.[(1)]

[1]Department of Cell Biology and Histology, [2]Departmant of Surgery, Radiology and Physic Medicine, Faculty of Medicine UPV/EHU, Leioa, Vizcaya, Spain

Introduction: Ultraviolet B (UVB) irradiation is responsible for a variety of dermal pathologies, ranging from sunburn or skin aging to cancer development [Schuch *et al.* 2014]. Molecular responses of skin to UV exposure are initiated by photochemical generation of reactive oxygen species (ROS) [Kim *et al.* 2015]. UV-induced ROS cause direct cell damage when levels increase above a certain threshold, which may be linked to cell senescence and wound-healing capacity [Toutfaire *et al.* 2017]. In this study we investigate, in an *in vitro* model, the effects of UVB on HFFs and ADSCs in ROS levels, senescence and cell migration.

Material and Methods: HFFs were cultured in DMEM, without phenol red, supplemented with 10% FBS, L-glutamine (4mM), sodium pyruvate (1mM) and penicillin-streptomycin (100U/ml). ADSCs were cultured in DMEM + Glutamax supplemented with 10% FBS. Both cultures were exposed to UVB irradiation (11-37 mJ/cm^2). Intracellular ROS levels were assessed using 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). The senescence phenotype was evaluated using β-galactosidase (SA-β-gal) activity. Cell migration was determined using a wound-healing model based on a two-well culture insert.

Results: Exposure of HFFs and ADSCs to UVB induced an increase in intracellular ROS levels and in the percentage of senescent cells and, also, a reduction in cell migration, in a dose-dependent manner. Although no significant differences were found in ROS levels between both cell types, UVB irradiation causes higher cell damage in HFFs, producing greater senescence and a decrease in cell migration when compared with ADSCs.

Conclusions: UVB exposure induces delayed damage in both cell populations but ADSCs show more resistance to such damage.

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CHANGES OF COLLAGEN FIBRES IN THE PLACENTAS OF PREGNANCY WITH LOWER EXTREMITY VENOUS INSUFFICIENCY

María J Álvarez-Rocha[1], Miguel A Ortega[1], Claudia Mesa-Ciller[1], Ángel Asúnsolo[2], Beatriz Romero[1], Juan De León-Luis[3], Melchor Álvarez- Mon[1,4], Julia Buján[1], Natalio García-Honduvilla[1].

[1]Departments of Medicine and Medical Specialities, Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares. Networking Biomedical Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain. [2]Department of Surgery, Medical and Social Sciences, Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares, Madrid, Spain. [3]Service of gynecology and obstetrics, Section of fetal maternal medicine, University Hospital Gregorio Marañón, Madrid, Spain. [4]Immune System Diseases-Rheumatology and Oncology Service, University Hospital Príncipe de Asturias, Alcalá de Henares, Madrid, Spain.

Introduction: Haemodynamic changes produced during pregnancy lead to elevated venous pressure in the legs and an increased resting consumption of oxygen. These events can cause varicose veins, or venous insufficiency (VI), which by creating an environment of hypoxia could affect the structure and function of the placental barrier. This study assesses the remodelling state of the placental villi by examining differences in collagens with a known role in villus structure and in placental barrier permeability between patients with and without VI.

Material and Methods: Samples of 67 placentas from women with VI (n=24) and without VI (n=43) during their pregnancy were processed for gene and protein expression analysis of COL-I, COL-III, MMP-2 and MMP-9 by RT-qPCR and immunohistochemistry.

Results: While no differences in COL-I expression levels were detected in the samples from women with and without VI, significant differences did emerge in both gene and protein expression levels of COL-III. Importantly, COL-I/III ratios were reduced in the VI group compared to controls. MMP-2 activity was similar in the two groups while MMP-9 levels were significantly elevated in VI with greatest expression differences observed at the level of the decidual cells.

Conclusions: Mothers who developed VI during pregnancy showed significantly higher COL-III and MMP-9 levels consistent with a state of remodelling of the placental villi.

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ASSESSMENT OF HEMATOLOGICAL PARAMETERS IN THE CATFISH *ARIOPSIS FELIS* IN ENVIRONMENTAL MONITORING

Misael Hernández Díaz[1], Edith Cortés Barberena[2], Marcela Galar Martínez[3], Irma Hernández Calderas[1], J. Ángel Vázquez Castro[1], Brian Real Huescas[1], J. Roberto Jerónimo Juárez[1], Xochitl Guzmán García[1].

[1]Departamento de Hidrobiología. División de Ciencias Biológicas y de la Salud. Laboratorio de Ecotoxicología, Histopatología. Universidad Autónoma Metropolitana Unidad Iztapalapa. Ciudad de México, México.
[2]Departamento de Ciencias de la Salud. División de Ciencias Biológicas y de la Salud. Laboratorio de Biología Celular y Citometría de Flujo. Universidad Autónoma Metropolitana Unidad Iztapalapa. Ciudad de México, México.
[3]Departamento de Farmacia. Escuela Nacional de Ciencias Biológicas. Laboratorio de Toxicología Acuática. Instituto Politécnico Nacional. Ciudad de México, México.

Introduction: Fish blood cells are sensitive to various environmental agents, responding with changes in their morphology and cytogenetic variations. The characterization and evaluation of blood cells package in fish as indicators can be useful in ecotoxicological studies and environmental monitoring programs. The aim of this study was to assess hematological parameters of blood cells in the catfish *Ariopsis felis* that can be included in protocols for the monitoring of the contamination.

Materials and methods: Ten specimens of catfish (*A. felis*) were collected in Tecolutla, Veracruz, Mexico. The fishes were anesthetized with 5% Benzocaine, recording weight (gr) and length (cm). Three ml of blood were extracted with herparinized syringes and subsequently 1: 100 dilutions were made for the erythrocyte count in the Nuebauer chamber with a manual hemocytometer. Blood smears were made and stained with Giemsa. Homogeneous zig-zag fields were observed and analyzed using optical microscope.

Results: Catfish average length and weight is 36 cm and 441 gr respectively, which means they are in adult state. Hematological parameters (number and morphology) in erythrocytes, polymorphonuclear and monomorphonuclear cells were evaluated. The erythrocyte count averaged 1.7-2.5 X 10^6 cel/mL. The morphology of erythrocytes, according to their nucleus-cytoplasmic relationship, had an ovoid shape and a well-defined nucleus, a size of 8-10 µm and membrane alterations (poquilocytosis), cytoplasm (Heinz bodies), nucleus (decondensed genetic material) and a less proportion lysis cells. In polymorphonuclears, granules were observed in cytoplasm and the nucleus occupies a greater proportion than the cytoplasm, its size was 10-15 µm. The monomorphonuclear cells measured between 7 and 10 µm and were observed with rounded and lobulated nuclei. Alterations were not found in polymorphonuclear and monomorphonuclear cells.

Conclusions: The catfish (*A. felis*) presents a cellular package with a well defined morphology. Hence why the evaluated hematological parameters as indicator of contamination is recomended in the monitoring protocols. The present study continues working on the establishment of pathological values of the cellular package in a special statistical R program.

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DIY HISTOLOGY: A NEW METHOD FOR DOING TISSUE MICROARRAY RECEIPT BLOCKS

Alejandro Seoane[1], Lucía García-Caballero[2], Vanesa Regueiro[3], Roberto Montero[3], Juan Cuevas[3], Rosalía Gallego[3]

[1]Department of Pathology, University Clinical Hospital, Santiago de Compostela, Spain.
[2]Department of Surgery and Medical-Surgical Specialties, School of Medicine and Dentistry, University of Santiago de Compostela, Santiago de Compostela, Spain
[3]Department of Morphological Sciences, School of Medicine and Dentistry, University of Santiago de Compostela, Santiago de Compostela, Spain.

Introduction. Tissue microarray (TMA) is the most useful method to compare multiple cells or tissue samples on the same slide. TMA is usually made transferring tissue cores from different donor blocks to a recipient one. This technique evolved from the multitumour sausage tissue block to TMA. Since the description of TMA punching paraffin tissue cores arranged in a Cartesian coordinate system by Kononen (*Nat Med 4:844,1998*) different methods were reported (Vogel. *Microarrays 3:103, 2014*) because commercial tissue arrayers are expensive. However, manual hole punching of the recipient block may result in block breakage, and the exact alignment of the holes is difficult. We present a new method to make easily the recipient blocks using a very common material in everyday life and in addition to very low cost.

Material and methods. Formalin fixed and paraffin embedded tissue samples from department of Pathology were used. The TMA was done using different materials, both home and laboratory source, according to the required thickness of the samples. Samples of 1-1.2 mm in diameter have used woodworking nails placed through lid tissue cassettes, and secured with paste FIMO®. This was used to make the recipient block as a routinely paraffin block and after paraffin has cooled, the nail matrix was removed by mechanical traction. For 2 mm samples we assume a paraffin block where performed equally spaced holes with a skin biopsy punch. We cover with liquid commercial silicone. Once has solidified get a mold that moulds of paraffin where only subtracted place from blocks donor tissue cylinders can be placed.

Results : This method of doing TMA recipient blocks is very low cost and easily reproducible allowing 100 sample cylinder of 1-1.2 mm in diameter. Using the biopsy punch and silicone the number of cylinders was lower (around 30). To make a negative of silicone mold with a hole diameter below 1.5 mm was difficult and in these cases the best option was the use of the nails.

Conclusion. This DIY method is very easy and allowed to study many tissue samples. No differences were observed when compared with commercial TMA builders.

DIMENSIONS AND RELATIONSHIPS OF THE DENTOGINGIVAL JUNCTION STRUCTURES IN HUMANS: MORPHOMETRICAL STUDY AND CORRELATIONS WITH GINGIVAL BIOTYPE

Andrea Cervera-Saiz[1], Rubén Salvador-Clavell[1], José Javier Martín de Llano[1,2], M. Fernanda Solá[3], Rubén Agustín[3], Amparo Ruiz-Sauri[1], Antonio Fons A.[3], Carmen Carda[1, 2, 4].

[1]Department of Pathology, Faculty of Medicine and Dentistry, University of Valencia, 46010 Valencia, Spain.
[2]Health Research Institute of the Hospital Clínico (INCLIVA), Valencia, Spain.
[3]Department of Stomatology, Faculty of Medicine and Dentistry, University of Valencia, 46010 Valencia, Spain.
[4]Ciber-BBN, Instituto de Salud Carlos III, Spain.

Introduction: Concept of thick gingival biotype suggests a wide and more resistant bone architecture to inflammation or trauma. Patients with thin biotype usually suffer gingival recession after a restorative intervention while thick biotype shows greater stability during remodeling. Our objective was to determine the range of dentogingival junction measurements that define each biotype.

Material and methods: A morphometric study was made in 26 samples obtained from 8 cadaveric donors. Samples were embedded in poly-methyl methacrylate, processed and stained with pH 3.5 and 8 toluidine blue and observed by optical microscopy. Images were merged with Photoshop and measured with AutoCAD. The morphological measurements were taken on the vestibular surface. Gingiva and alveolar bone were measured from the tooth drawing lines perpendicular to its axis, obtaining 9 measures for each sample. Based on this, samples were classified by biotype. This classification was compared with another established by dentists based in visual parameters. Finally, a radiographic study was made considering the same morphological measurements.

Results: Differences between samples were clear, so it was possible to classify those according different biotypes. Cases 5th and 7th belonged to the thick gingival biotype (i.e., gingiva measured at the bone crest level was 2.60 mm or higher), 1st, 2nd, 4th and 6th to the intermediate biotype and 3rd and 8th to the thin biotype.

MORPHOMETRICAL STUDY		
Thin	Intermediate	Thick
Case3	Case1 (high intermediate) Case8 Case2	Case5 Case7

Comparing to the visual study, a clear coincidence was obtained in the cases with thick biotype, whereas for thin and intermediate biotypes it was more complicated to establish an agreement. In the radiographic study, 6th was considered thin.

Conclusions: The thick biotype was the easiest to identify. Although, it turned out more difficult to establish the separation between thin and intermediate biotypes. It must be taken into account that samples were from cadaveric donors and conserved with formaldehyde so some variability could be introduced by this treatment. We are currently increasing sample number to cope with this variability.

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DISTRIBUTION OF HIGH ENDOTHELIAL VENULES

Benedicto Molina Espinoza[1], Enrique Olave Riffo[1]

[1]Department of Basic Sciences, Faculty of Medicine, University of La Frontera, Chile

Introduction: The lymphoid system provides a line of defense against invasive pathogens constituting a barrier that is in charge of the immune defense of the organism, constituted by lymphocyte accumulations. Some of them are limited by a capsule of connective tissue and others not encapsulated. Their cells constitute the functional components of the innate and adaptive immune system. This set of lymphatic organs are distributed throughout the human body. In the lymphatic nodes, encapsulated organs exposed to the lymph circulation in their area of the deep cortex have been described high endothelium venules, called the postcapillary venules where the lymphocytes leave the blood vessel through the cytoplasm of these vascular cells through emperipolesis to present antigens and assemble an immune response.

Material and Method: The objective of this study is to determine if these venules are exclusive to the Lymph Nodes. We studied 20 tonsils surgically extracted from patients with chronic tonsillitis and 30 lymph nodes in the esophageal mucosa, which were fixed by immersion in 10% formalin buffer and treated with traditional dehydration and inclusion techniques in Paraplast, obtaining serial cuts of 7 µm of thickness and dyed with current method for optical microscopy.

Results: In all the tonsils and lymph nodes analyzed in their perinodular and marginal regions were found the presence of postcapillary veins with cubic endothelium.

Conclusions: We conclude that the passage of lymphocytes to present antigens by emperipolesis also occurs In non-encapsulated lymphoid organs.

Acknowledgements: Department of Pathological Anatomy, Faculty of Medicine, University of La Frontera, Temuco, Chile.

ECTO-NUCLEOTIDASES AS POTENTIAL BIOMARKERS FOR DIAGNOSIS OF ENDOMETRIOSIS

Carla Trapero[1], August Vidal[1,2,3], Lluís Jover[4], Mª Eulalia Fernández Montolí[2,5], Amparo García Tejedor[2,5], Jordi Ponce[2,5], Francesc Tresserra[6], Pere Barri[6], Buenaventura Coroleu[6], Mireia Martín Satué[1,2].

[1]Departament de Patologia i Terapèutica Experimental, Facultat de Medicina i Ciències de la Salut, Campus Bellvitge, Universitat de Barcelona, Spain
[2]Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), Spain
[3]Servei d'Anatomia Patològica, Hospital de Bellvitge, Barcelona, Spain
[4]Departament de Salut Pública, Facultat de Medicina i Ciències de la Salut, Campus Bellvitge, Universitat de Barcelona, Spain
[5]Servei de Ginecologia, Hospital de Bellvitge, Barcelona, Spain
[6]Hospital Universitari Dexeus, Barcelona, Spain

Introduction: Endometriosis is an inflammatory disorder characterized by the growth of endometrial tissue in extrauterine locations. Ovarian endometriomas are a common form of endometriosis. Late diagnosis is the main problem in this pathology; thus, it is important to identify clinical biomarkers. Ecto-nucleotidases are enzymes that hydrolyze extracellular ATP to adenosine and are involved in many inflammatory processes. Alterations of ecto-nucleotidase activity are related to inflammatory disease states.

The aims of the present project were: I) to characterize the expression and activity of ecto-nucleotidases in ovarian endometriomas, and II) to evaluate the utility of ecto-nucleotidases expression in the contents of endometriomas as a possible biomarker of endometriosis.

Material and Methods: To achieve our objectives, ovarian endometrioma tissue were analysed through immunolabeling and nucleotidase activity assays (objective I).

Echo-guided aspirated fluids of ovarian endometriomas and simple ovarian cysts were studied with ELISA technique (objective II). A case-control comparative study was conducted with two groups: a) fluid content of endometriomas from women with endometriosis and b) fluid content of simple cysts from women without endometriosis. The expression of adenosine deaminase (ADA), alkaline phosphatase (ALP), and ecto-nucleotide pyrophosphatase/phosphodiesterases 1 and 3 (ENPP1 and ENPP3) was determined in fluid of ovarian endometriomas and simple cysts.

Results: We showed that ecto-nucleotidases are present in ovarian endometrioma tissue. There was a differential distribution of ecto-nucleotidases among epithelia and stromal cells of ovarian endometriotic lesions. The case-control comparative study showed a significant difference in ADA and ENPP1 levels among endometriomas aspirates in comparison to simple cysts ($p<0.001$). Comparisons of ALP and ENPP3 levels among endometriomas and simple cysts did not turn up significant differences ($p>0.05$).

Conclusions: Ecto-nucleotidases are abundantly present in ovarian endometriotic cells, and alterations of their activity may be related with endometriosis development. Furthermore, ADA and ENPP1 are biomarker candidates of endometriosis. Our results emphasize the relevance of studying purinergic signaling in endometriosis in order to add to the knowledge of physiopathological mechanisms underlying this pathology.

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IMMUNOLOCALIZATION OF SONIC HEDGEHOG IN EMBRYO-FETAL MOUSE (*Mus musculus*) DEVELOPMENT

Daniel Conei Valencia[1,2,3,4], Gustavo Saint-Pierre Contreras[1], Mariana Rojas Rauco[1].

[1] Laboratory of Comparative Embryology, Anatomy and Developmental Biology Program, Institute of Biomedical Sciences, Faculty of Medicine, Universidad de Chile, Chile.
[2] Doctoral Program in Morphological Sciences, Faculty of Medicine, Universidad de La Frontera, Temuco, Chile.
[3] Department of Morphological Sciences, Faculty of Science, Universidad San Sebastián, Puerto Montt, Chile.
[4] Conicyt scholar. CONICYT, PFCHA/Doctorado Nacional/2017-21170693.

Introduction: Sonic hedgehog (SHH) is an essential morphogen for the development of various structures, such as notochord, floor plate of neural tube, limbs, among others. We sought to determine the immunolocalization of SHH in mouse embryos and fetuses.

Material and methods: Ten pregnant mice were euthanized, one group of 5 animals at 12 days postcoital (p.c.), and another group at 17 days p.c. The embryos and fetuses obtained were fixed in formalin buffered in PBS and included in paraplast. Serial cross sections were made. Polyclonal SHH antibody (Santa Cruz Biotechnology, H-160, rabbit), dilution 1:100 was used. The immunolocalization of the positively-labeled samples was identified.

Results: SHH expression in the 12 days p.c. was immunopositive in notochord, neural tube floor plate, radioactic pre-articular ulna, and practically all epithelia: bronchial, intestinal, bladder and urethra. In fetal stage, at 17 days p.c. The immunopositivity disappears in the cartilage except for areas of ossification, decreases in the epidermis but appears in hair follicles. The intestinal mucosa has been differentiated into segments, showing a greater immunostaining at the level of intestinal villi.

Conclusions: SHH acts in different stages of the gestational period, being key in the differentiation of different structures. In embryonic stages, it is vital in the formation of the nervous system, organogenesis and limb formation, so its expression is found in these areas. However, in the fetal stage the expression changes to more specialized structures such as hair follicle and intestinal villi.

Keywords: Sonic hedgehog, morphogen, organogenesis, immunohistochemistry.

HISTOLOGICAL ANALYSIS OF THE ORIGIN AND DEVELOPMENT OF SOMATIC EMBRYOS FROM LEAVES AND APEX EXPLANTS OF *QUERCUS* SPECIES

Elena Corredoira, MªTeresa Martínez, MªJosé Cernadas, Mª del Carmen San José

Group of Biotechnology and Forestry Improvement. Instituto de Investigaciones Agrobiológicas de Galicia. IIAG-CSIC. Avda. de Vigo s/n. 15705 Santiago de Compostela, Spain.

e-mail: elenac@iiag.csic.es

Introduction: Somatic embryogenesis (SE) constitutes an important tool for plant regeneration and for investigating the mechanisms regulating embryo formation. Despite its potential value in tree breeding strategies, SE is still rather unsuccessful in *Quercus* species, as factors responsible for the initiation of embryogenesis, including the presence of embryogenic nodular structures and development of somatic embryos from mature material, remain largely unknown. The purpose of the present study was therefore to identify the cells involved in the formation of somatic embryos from leaf and shoot apex explants derived from adult *Q. robur* and *Q. alba* trees.

Material and Methods: The most apical expanding leaf and shoot apex explants were excised from in vitro stock shoot cultures, and the explants were then cultured in induction medium consisting of Murashige and Skoog (1962) mineral salts and vitamins, 500 mg/L casein hydrolysate, 6 g/L Vitroagar, 30 g/L sucrose, 21.48 µM naphthaleneacetic acid and 2.22 µM 6-benzylaminopurine. Leaf and apex explants were collected after culture for 2, 4, 6, and 8 weeks on induction medium and were processed in paraffin or plastic-embedding protocols.

Results: Embryogenic nodular structures mainly formed on the callus generated on the abaxial side of leaf explants, especially in the thickening midvein and the margins of the leaf blade. In shoot tip explants, embryogenic structures mainly developed on the abaxial side of the outermost leaf primordia and in the small callus formed in axial zones. These embryogenic nodular structures comprised small undifferentitated cells and meristematic cells. The indirect origin of somatic embryos involves cell dedifferentiation and proliferation, with subsequent formation of proembryo cell aggregates, which generally develop in association with the presence of polyphenol-rich cells.
Somatic embryos developed asynchronously following the typical stages: globular, heart, torpedo and cotyledonary. Well-developed somatic embryos had both shoot and root meristems, with procambial strands between the shoot and root apices. Anomalous morphologies, mainly affecting the number of cotyledons, were also observed and included multiple or aborted cotyledons and embryos with fused cotyledons.

Conclusions: This research contributes to the identification of cells involved in the somatic embryo induction process in leaf and apex explants of two oak species and improves our understanding of SE.

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EVALUATION OF PHENOLIC PRODUCT IN THE PREVENTION OF INDUCED NEPHROPATHY

Paulo Pardi[1], Lucineia Santos[2], Reginaldo Santos[2], Jose Quincoces[2], Ivair Donizetti[2], Enrique Olave[3]

[1] Faculdade Anhanguera, Sao Paulo, Brasil. Colaborate Professor in MSc., Morphology area,Universidad de La Frontera, Chile
[2] Universidade Anhanguera de Sao Paulo, Brasil
[3] Professor in MSc., Morphology area, Facultad de Medicina, Universidad de La Frontera, Chile.

Introduction. The incidence of nephropathy by contrast has increased as a result to use for diagnostic purposes and therapeutic intervention. Its incidence in the general population is low, but it increases exponentially in patients with risk factors such as diabetes and previous renal disease. Several protocols have been used in the attempt to prevent contrast-induced nephropathy, such as hydratation with physiological solution, use of low osmolality contrast media, infusion of sodium bicarbonate and n-acetylcysteine.These last have been considered the most effective.The objective of this study was determine the role of the phenolic compound (PhP) on renal protection in albino Wistar rats compared to the use of hydration and n-acetylcysteine.

Material and Method. The Wistar rats were divided in 5 randomized groups and treated during 5 days before, N-acetylcysteine group (300 mg/ day), PhP group (100 mg/day), hydratation group (100 ml/day), physiological solution (100 ml/day), and control group; all substances were administered by gavage. Following these procedures, the animals were induced to experimental nephrotoxicity, receiving indomethacin (10 mg/kg, intraperitoneally, ip), and 15 min after one injection of N-nitro-L-arginine methyl ester (L-NAME, 10 mg/kg, ip), and after the contrast agent (Ultravist - meglumine amidotrizoate), 6 ml/kg by the ip route. After 24 hours the animals were sacrificed and biochemical and morphological analyzes were performed.

Results. The results showed that the biochemical levels of urea and creatinine and the morphology results in the group treated with PhP remained normal compared to the control group; aspects of tubular necrosis and glomerular atrophy have already been found in the hydration n-acetylcysteine group, with small glomerular atrophy in the hydrated group.

Conclusions. In general for the model used the preventive use of the PhP compound was effective and presented results of nephroprotection after the use of contrast media.

HEMINASAL HISTOLOGY OF THE COMMON VAMPIRE BAT *Desmodus rotundus*.

Hector Serrano[2], Saúl Gaona-Domínguez[2], Maria Dolores García-Suárez[1], J. Ignacio Olave-Leyva[3], Mariana Mendoza-Castillo[2], Jose Luis Gómez-Olivares[2], Juan Ocampo-López[3].

Departaments of [1]Biology, and [2]Health Sciences, Metropolitan Autonomous University, Iztapalapa Campus, Mexico City, Mexico.

[3]Laboratory of Histology and Histopathology. Veterinary Medicine and Zootechny Academic Area, Agropecuary Sciences Institute, Autonomous University of the State of Hidalgo, Tulancingo de Bravo, Hidalgo, Mexico.

Introduction. Vampire bats are effective zoonotic and human infection vectors. *Desmodus rotundus* is the most conspicuous aerial rabies virus vector to human and domestic animals. Nostrils are deep and prominent containing common structures like the vomeronasal organ (VNO) and the mitral olfactory epithelium (MOE) both having neuronal ends that effectively conducts the stimuli elicited by volatile compounds that regulate social and behavioural aspects of the organism. Scarce and controversial studies have been reported on the VNO histology. The goal of this study was to gain knowledge on the histology of the VNO in bats, particularly on *Desmodus rotundus*.

Material and Methods. Male and female *D. rotundus* individuals were captured on the hill mountain region of Hidalgo, México and transported to the lab where animals were euthanized by sodium pentobarbital overdose. Skull was obtained and fixed for 2 days in buffered formalin solution. Longitudinal sections were processed for routine HE studies on paraffin embedded tissues. Histology preparations of 4 µm sections were analysed by light microscopy.

Results. Anatomically, the various components of the heminasal region of D. rotundus show a different distribution as adaptations to the flight and hematophagous habits. As in other mammals, MOE and VNO are present in this bats and their respective neuronal connections and prolongations allows the initial steps of odour stimulation. VNO and MOE can be differentiated by the histological characteristics (Figure 1). In the first case, it is possible to delimitate the terminal neurons responsible for pheromone-initiated stimulation that renders the behavioural aspects of reproduction and socialization.

Conclusions. Besides the contradictory reports found in the literature, *Desmodus rotundus* show a distinctive VNO that has been relocated as an adaptation to the animal habits. The morphological characteristics resemble those found in rodents but having characteristics that optimizes the reception of volatile compounds that elicits different social, sexual and behavioural responses. The most conspicuous characteristic is the apparent neuronal development in D. rotundus.

SCANNING ELECTRON MICROSCOPY STUDY OF IN VITRO PROPAGATED *Epidendrum radicans* PAV.EX LINDL.

Hector Serrano[4], Y. Odemaris Buendía López[1], Saúl Gaona Domínguez[4], Alondra Castro Campillo[1], Arturo Salame Méndez[3], José Luis Gómez Olivares[4], J. Angel Lechuga-Corchado[3], Claudia Barbosa-Martínez[1], María Dolores García-Suárez[1].

Departaments of [1]Biology, [2]Biology of Reproduction, [3] Biotechnology, and [4]Health Sciences. Metropolitan Autonomous University, Iztapalapa Campus, Mexico City, Mexico.

Introduction. *Epidendrum radicans* Orchidaceae, the fire star, is a native American tropical a subtropical orchid capable to flowering several times a year. *In vitro* micropropagation is an option to overcome disadvantages for its massive production and possible commercialization. However, seed germination process is poorly understood. The goal of this communication is to gain knowledge about the initial steps of seed germination and follow them at the scanning electron microscopy level.

Material and Methods. Seeds were obtained from a local nursery at México City, length and width measured, and viability determined by using a modified Lakon test. Embryos were cultured in Knudson C Medium, supplemented with agar, sugar with no plant regulators. Different germination stages were sampled and processed for SEM where embryo and testa were analysed and their growth was classified.

Results. The seeds are formed by embryo and testa with no endosperm inside. We use a 5 different growth size category classification: hydrated seeds, testa-enclosed protocorm, germinated embryo (testa-free protocorm), plantlets with foliar primordium and plantlets with leaves. Structure and organization of *in vitro* seed cultured *Epidendrum radicans* were observed under SEM. True embryo growth and differentiation was characterized by the presence of undifferentiated isodiametric cells and elongated differentiated cells on meristematic zones where future leaves will be formed. Fertile seeds for E. radicans, have low viability and decreasing with time. In only six months, at the better storing conditions, viability decreases from 24.44% to 4.37%. Under simple culture condition, *E. radicans* seeds do germinate but the efficiency is extremely low and on the tested medium, a germination index of 28% was obtained.

Conclusions. Under simple germination conditions, besides the low germinative activity of E. radicans, it is possible to obtain and analyse under SEM the germinative process. This work show different structural aspects of embryo and testa of an Orchid and their different growth and developmental stages. The reticulate structure of testa and the differential characteristics of meristematic embryo cells as well as their differentiation into primordial leaf structures could give strong contributions to the *in vitro* germination analysis and to promote *Epidendrum radicans* propagation.

HISTOLOGICAL AND ULTRASOUND CORRELATION OF SKELETAL MUSCLE CHANGES IN EXPERIMENTAL MYOTENDINOUS INJURY

Ignacio Ruz-Caracuel[1,2], Julia Casado-Ruiz[1], Fernando Leiva-Cepas[1,2,4], Miguel Jiménez-Fermín[3], Ignacio Jimena[1,2,4], José Fernando Jiménez-Díaz[3], José Peña [1,2,4].

[1]Department Morphological Sciences, Section of Histology, Faculty of Medicine and Nursing, University of Córdoba, Spain.
[2]Research Group in Muscle Regeneration, University of Córdoba, Spain.
[3]Laboratory of Performance and Sports Readaptation, Faculty of Sport Sciences, University of Castilla-La Mancha, Spain.
[4]Maimónides Institute for Biomedical Research IMIBIC, Reina Sofia University Hospital, University of Cordoba. Spain.
** Present address: Department of Pathology. La Paz University Hospital, IDIPAZ. Madrid.Spain.*

Introduction: Ultrasound is capable of providing useful real time feedback on the presence of pathologic changes in muscle. The aim of this study is to correlate ultrasound findings with histological muscle fibers changes taking place after an experimental model of tendon injury in rats.

Material and Methods: 28 Wistar rats were injuried by triceps sural tendon compression and were studied 1, 3, 6, 10, 15 and 20 days post-injury. Before the animals were sacrificed, the gastrocnemius muscles were evaluated by ultrasonography. Muscles were extracted immediately for histological and histochemical studies.

Results: During the first six days we observe edema and inflammatory infiltrate. Histological findings show that there are statistically significant differences among the percentages of abnormal fibers in the different post-injury groups. On days 10, 15 and 20 a large number of fibers were observed showing important cytoarchitectural changes with NADH-tr histochemical reaction suggestive of a process of muscle fibers repair. Sonography shows an increase of echogenecity the 1st and 3rd day post-injury, which decreases progressively until day 15th. Echogenecity day 20th post-injury is completely normal.

Conclusions: Results suggest that tendon compression leads to cytoarchitectural changes in muscle fibers. Sonographic diagnosis is not specific of this lesion but could be helpful in its follow-up and evolution.

EXTRACTS OF DENERVATED AND REGENERATIVE MUSCLES INCREASE THE NUMBER OF PERICYTES IN SKELETAL MUSCLES OF NORMAL MICE

Ignacio Ruz-Caracuel[1,2], Rubén Giovanetti-González[1], Fernando Leiva-Cepas[1,2,3], Juan Cámara Pérez[1], Evelio Luque[1,3], Ignacio Jimena[1,2,3], José Peña[1,2,3]*

[1]Department Morphological Sciences, Section of Histology, Faculty of Medicine and Nursing, University of Córdoba, Spain.
[2]Research Group in Muscle Regeneration, University of Córdoba, Spain.
[3]Maimónides Institute for Biomedical Research IMIBIC, Reina Sofia University Hospital, University of Cordoba. Spain.
** Present address: Department of Pathology. La Paz University Hospital, IDIPAZ. Madrid.Spain.*

Introduction: Pericytes may play a key role in myogenesis of skeletal muscle tissue. We aimed to test whether the administration of muscle extract with myogenic potential induces activation of the population of pericytes so that they contribute to the renewal and maintenance of satellite cells and participate in the processes of muscle growth and regeneration.

Material and Methods: 22 transgenic mice of C57B/L6 strain were divided into four groups, two of them were treated for 5 days with 0.5 ml/day intraperitoneally of denervated muscle extract and regenerative muscle extract respectively. After slaughter we took the rear muscle mass and studied the soleus muscle, which was analyzed morphometric and quantitatively using imaging software. Finally we performed statistical analysis.

Results: Our data show that both types of extract produced a statistically significant increase in the number of positive alpha smooth muscle actin cells and positive alkaline phosphatase profiles, unlike the control group and the group treated with vehicle. This suggests a likely myogenic response of pericytes. Histological, histochemical and immunohistochemical analysis show that satellite cells (MyoD+) have also an activation response.

Conclusions: Muscle extracts stimulate the population of positive alpha smooth muscle actin and alkaline phosphatase cells (compatible with pericytes) and activate satellite cells in the absence of injury. This model could be of clinical interest to stimulate myogenesis in a targeted manner in pericytes.

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MERKEL CELLS OF HUMAN ORAL MUCOSA EXPRESS THE PLURIPOTENT STEM CELL TRANSCRIPTION FACTOR SOX2

Lucía García-Caballero[1], Javier Caneiro[2], Noel González-Ortega[2], Dora Ínsua[2], María Otero[3], Manuel Collado[3], Andrés Beiras[4], Rosalía Gallego[4]

[1]Department of Surgery and Medical-Surgical Specialties, School of Medicine and Dentistry, University of Santiago de Compostela, Santiago de Compostela, Spain
[2]Department of Pathology, University Clinical Hospital, Santiago de Compostela, Spain.
[3]Health Research Institute of Santiago, Santiago de Compostela, Spain.
[4]Department of Morphological Sciences, School of Medicine and Dentistry, University of Santiago de Compostela, Santiago de Compostela, Spain.

Introduction. Friedrich Sigmund Merkel described in 1875 a type of epidermal cell with a specific inervation, that was called “Tastzellen” to indicate its tactile function. Merkel cells are localized primarily in the epidermis of fingertips, but also in hair follicles, lips, eyelids and oral mucosa. SOX2 is a key transcription factor iimportant in the maintenance of embryonic neural crest stem cell pluripotency. SOX2 was described in Merkel cells of skin, but only one paper showed a photograph of SOX2 in Merkel cells of oral mucosa (lip) (Laga et al., Am J Pathol 2010; 176:903-13). The aim of the present work was to analyze the expression of SOX2 in Merkel cells of human oral mucosa of different regions.

Material and methods. Normal samples of human oral mucosa were obtained from the files of the Pathology Department of the University Clinical Hospital of Santiago de Compostela. The samples analyzed (n=15) corresponded to mucosa of lip, gum, cheek and palate. Immunohistochemistry was automatically performed using an AutostainerLink 48 immunostainer (Dako-Agilent, Carpinteria, CA). Briefly, the slides were consecutively incubated at room temperature in: 1) SOX2 rabbit monoclonal antibody (Novus Biologicals, Littleton, CO) at 1:5000 for 20 min or in cytokeratin 20 (CK20) mouse monoclonal (Dako-Agilent), RTU for 20 min; 2) EnVision FLEX/HRP (dextran polymer complex) for 20 min; 3) chromogen solution (DAB) for 10 min; and 4) EnVision FLEX hematoxylin for 9 min. Double IF: SOX2 ab 1:4000 48h 4ºC; donkey anti-rabbit 1:200 1h RT; CK20 Dako-Agilent 15 min RT; sheep anti-mouse (Sigma) 1:200 1h RT.

Results. SOX2 immunoreactivity was found in the nuclei of some oral mucosa cells. The region with higher density of positive cells was gum. Immunostained cells were primarily localized at the tips of rete ridges and appeared isolated or forming small clusters. Serial sections immunostained for SOX2 and CK20 showed coexpression of both markers, proving that SOX2 positive cells in oral mucosa were Merkel cells. Double immunofluorescence confirmed that virtually all oral Merkel cells expressed SOX2. SOX2 in oral Merkel cells may play a role in Merkel cell differentiation and could support the theory of a neural crest origin for these cells.

Conclusion. SOX2 immunoreactivity was found in the nuclei of virtually all Merkel cells localized in oral mucosa. The region of oral mucosa with higher density of Merkel cells was gum. Further research is warranted to ascertain the precise role of SOX2 in oral Merkel cells.

ULTRASTRUCTURAL CHARACTERISTICS OF GLIAL AND INTERSTITIAL CELLS OF CAJAL IN THE LIZARD INTESTINE: PRESENCE OF PRIMARY CILIUM

Javier Gracia LLanes, Marta Monzón, Pablo Iruzubieta, Carmen Berga, Mª José Luesma, Mª Concepción Junquera.

Faculty of Medicine, Department of Human Anatomy and Histology, University of Zaragoza / Institute for Health Research Aragón (IIS), Domingo Miral s/n, 50009 Zaragoza, Spain

Introduction: In mammals, enteric nervous system consists of neurons and glial cells which are mainly organized in two ganglionated plexuses: the myenteric plexus, and the inner and outer submucosal plexuses. Enteric glial cells establish a direct contact with neurons; their cytoplasm prolongations surround the unmyelinated axons. The interstitial cells of Cajal (ICCs) are located around enteric ganglia and in both muscular layers forming an interconnected network. It is now known that some ICCs and glial cells in mammals present a single cilium but its presence in the rest of vertebrate classes has been not still demonstrated. The aim of the present study is to characterize, at the ultrastructural level, the enteric glial cells and ICCs in the lizard intestine.

Material and methods: We used ten adult lizards *Podarcis hispanica* (Reptilia). Small and large intestine wall samples were fixed with 2.5% glutaraldehyde in PB buffer (pH 7.3) and routinely processed for TEM visualization.

Results: Lizard intestinal ganglia show a lower number of neurons than those from mammals. Glial cells were smaller than neurons and they showed dark nuclei because of their condensate heterochromatin. These glial cells surround axons mainly containing mixed both cholinergic and peptidergic vesicles. Occasionally, some axons were found surrounded by myelin sheaths. We found ICCs around enteric ganglia of the myenteric plexus. They present triangular or spindle forms and a very voluminous nucleus, with scarce marginal heterochromatin, surrounded by a thin perinuclear cytoplasm that expands with long cytoplasmic processes. ICC processes penetrate and connect with other ICCs located in the connective tissue of the both muscle layers by gap-like junctions forming a three-dimensional network. In addition, we demonstrate the presence of a primary cilium in ICCs as well as in glial cells. We describe their ultraestructural features: basal foot and cap in the basal corpuscle, (9+0) axonema, and a characteristic domain of membrane: the ciliary pocket.

Conclusions: Our data support that the single cilium is present in both kinds of cells and consequently, this is a phylogenetically preserved structure.

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MORPHOLOGICAL STUDY OF PECTEN OCULI IN THE YELLOW-LEGGED GULL (*Larus michahellis*)

Yolanda Segovia, Noemí Victory, Alicia Navarro-Sempere, M. Magdalena García

Department of Biotechnology, Faculty of Sciences, University of Alicante, Spain

Introduction: The pecten oculi is a highly vascularized and pigmented organ that overlies the optic disc and projects into the vitreous body of the avian eye. Although this structure is well known for more than a century, its functions are still a matter of debate. Both formation of a blood-retina barrier and nourish the avascular retina are the most plausible functions. Others functions are: to increase visual precision, protect the blood vessels from harmful beams of the ultraviolet light as well as oxygen radicals, increased metabolic rate to optimize eye physiology in low temperatures at high-altitude flights. Moreover, functional morphology of the pecten correlates with the life-style of the bird. The goal of this study is to describe this organ in the yellow-legged gull (*Larus michahelis*) using light and electron microscopy.

Material and Methods: Study was carried out from 6 pecten oculi belonging 3 seagulls. The eyeballs were cut at the equator, and two posterior eyes which contained the pecten oculi was photographed using digital camera attached stereomicroscope (Leica). Three pecten oculi were fixed in 10% buffered formalin for histological procedure and blocked in paraffin. Sections were stained using hematoxilin and eosin and others staining techniques. Three more pectin oculi were fixed in 1% paraformaldehyde and 1.6% glutaraldehyde in phosphate buffer solution. One of them were processed for scanning electron microscopic and two for transmission electron microscopy examination. The diameter of the capillaries and thickness of capillary basement membrane of pecten oculi were measured with image J program.

Results: This pecten oculi is pleated and consists of 18- 20 pleats. Capillaries, small blood vessels (afferent and efferent) and pigmented stromal cells can be identified. Endotelial cells display numerous microvillous-like folds projecting from their internal (luminal) and external (ablaluminal) surfaces. Each capilar is rounded by fibrous, PAS-positive basal lamina, which also encloses pericytes. Intercellular spaces between capillaries are occupied by pigmented stromal cells which show long membrane projections and large granules.

Conclusions: The pecten oculi of *Larus michahellis,* a water bird hunting diurnally, are pleated and similar with other diurnal birds.

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LOCALIZATION OF THE ACROSOME PROTEIN ZONA PELLUCIDA SPERM-BINDING PROTEIN 3 RECEPTOR (sp56) IN THE *Gopc*$^{-/-}$ MOUSE SPERMIOGENESIS

Maider Bizkarguenaga[1]; Laura Gómez-Santos[1]; Juan Francisco Madrid[2]; Francisco José Sáez[1] and Edurne Alonso [1].

[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, School of Medicine, University of Murcia, IMIB-Arrixaca, Murcia, Spain

Introduction: The acrosome is an essential organelle containing hydrolytic enzymes that allow the spermatozoon to penetrate the oocyte during fertilization. The acrosome is properly formed during the spermiogenesis. However, a single mutation in one specific gene, as *Gopc*, can cause globozoospermia. In this type of teratozoospermia the acrosome development is deficient because of the incorrect fusion of pre-acrosome vesicles and the incorrect assembly of the cytoskeleton, resulting in round-headed spermatozoa. *Gopc*$^{-/-}$ mice are used as animal models for the study of globozoospermia *in vivo*.
Sp56 (Zona pellucida sperm-binding protein 3 receptor) is an acrosome protein with an important role during fertilization. It is suggested that this protein binds to the ZP3 glycoprotein of the zona pellucida of the oocyte, triggering the acrosome reaction. Firstly localized in the plasma membrane, nowadays it has been described in the acrosomal matrix. It is synthesized in the Golgi apparatus and post-transcriptionally modificated during spermiogenesis.

Material and methods: The expression of sp56 in wild type mice and *Gopc*$^{-/-}$ mice was analyzed by Western Blot on testis extracts. Furthermore, it was immunohistochemically localized with anti-sp56 on Bouin fixed and paraffin-embedded testis sections. DAB was used as chromogen, and sections were counterstained with Hematoxilin.

Results: Sp56 protein was localized not only in the acrosomal matrix, but also in the cytoplasm of spermatids during spermiogenesis, from step 1 to step 12 spermatids. However, towards step 13 spermatids, the staining was exclusively related to the acrosome. There was no staining in the meiotic cells, neither in the Sertoli cells. The same pattern was observed in both wild type and knockout mice, as well as in the Western Blot analysis.

Conclusions: Sp56 has been described as a fertilization protein because of its localization in the acrosome; however the described localization in the cytoplasm of the haploid cells may suggest that it could have another synthetic pathway, different from the Golgi apparatus. We can also suggest that the deficient acrosome development of *Gopc*$^{-/-}$ mice do not interfere in the Sp56 synthesis.

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A METHOD FOR EMBEDDING HARD SUBSTANCES ALLOWING HISTOLOGICAL STUDIES FROM MACROSCOPIC TO ULTRASTRUCTURAL LEVEL

M. J. Gayoso[1], J. Gayoso[1], M. Garrosa[1], P. Jiménez[2], T. Girbés[2] and S. Gayoso[1].

[1]Department of Cell Biology, Histology and Pharmacology. Faculty of Medicine. INCYL. University of Valladolid. Spain.
[2]Department of Nutrition and Bromatology.Faculty of Medicine. University of Valladolid. Spain.

Introduction: The hard tissues, for example cartilage or bone, have difficulties for their histological study basically due to embedding and cutting. The development of prostheses and implants, most of them containing hard substances including metals as titanium, often requires histological studies in the experimental phase as well as in the analysis of the generated interactions. The classic embedding technics in mixtures of paraffin, celloidin or synthetic resin do not produce good results in hard tissue studies. glycol methacrylate and their derivatives, after grinding and polishing, allow getting hard tissues slices of 5 to 10 µm thick, although it is a difficult and laborious method.

Material and Methods: We use Wistar albino rats under the European Community and Spanish care dispositions. These animals received a mandible osteotomy of 4mm in diameter which was covered with a titanium 12.7 µm thick foil. After transcardial perfusion with the selected fixative (paraformaldehyde or paraformaldehyde-glutaraldehyde) the whole mandibles were embedded in low viscosity epoxy-resin without decalcification. After polymerization the mandibles were sectioned using a precision saw IsoMet™ 4000 (Buehler).

Results: The sections (about 300 µm thick) are useful to be studied with low power microscopy (1 to 100 x). Histochemistry as von Kossa and alizarin red stains can be done on these thick sections. Also thick sections can be morphologicaly studied with SEM. and find out the atomic composition of the interest area using Backscattered Electron SEM. Parts of the thick sections were re-embedded in epoxy-resin to obtain semithin (1 to 2 µm) sections for high resolution light microscopy (to 1200 x) or, after deplastification, for other special staining. On these pieces we selected again the interesting area to obtain ultrathin (about 100 nm) sections to be observed under TEM (to 400.000 x).

Conclusions: This method is relatively simple, and very useful in morphological studies because allows an absolute magnification range.

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MESH FIXATION WITH TWO DIFFERENT SURGICAL GLUE APPLICATION FORMS IN AN *EX VIVO* AND *IN VIVO* STUDY OF ABDOMINAL WALL REPAIR

Gemma Pascual[1], Claudia Mesa-Ciller[2], Bárbara Pérez-Köhler[2], Marta Rodríguez[2], Ángel Ortillés[3], Estefanía Peña[4], Begoña Calvo[4], Juan M. Bellón[2].

[1]Department of Medicine and Medical Specialities. Faculty of Medicine and Health Sciences, University of Alcalá. Networking Research Centre on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN). Alcalá de Henares, Madrid, Spain.
[2]Department of Surgery, Medical and Social Sciences. Faculty of Medicine and Health Sciences, University of Alcalá. Networking Research Centre on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN). Alcalá de Henares, Madrid, Spain.
[3]Aragón Institute of Engineering Research (i3A). Department of Animal Pathology. University of Zaragoza. Networking Research Centre on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN). Zaragoza, Spain.
[4]Aragon Institute of Engineering Research (i3A). University of Zaragoza. Networking Research Centre on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN). Zaragoza, Spain.

Introduction: Nowadays, the use of different adhesives for the mesh fixation in hernia repair is increasing mainly because their ability to reduce chronic pain and tissue damage in the patient. The main aim of this study was to assess the adhesive properties and behavior of a medium-chain (n-butyl) cyanoacrylate glue, through a biomechanical and histological study, comparing this method with the suture fixation.

Material and Methods: New Zealand white rabbits were used for *ex vivo* and *in vivo* mechanical testing. Two pieces of polypropylene mesh were implanted over a healthy abdominal tissue and partial abdominal wall defect, respectively. Depending on the fixation method used, four groups were established: Glue-Drops (n=12) and Glue-Spray (n=12), where cyanoacrylate glue was chosen to attach the meshes to the abdominal wall, and Suture-Simple interrupted (n=12) and Suture-Continuous (n=12), where the meshes were fixed with a polypropylene suture. The *in vivo* groups were tested after 14 days of the mesh implantation, and the *ex vivo* groups immediately. An additional histological study was performed in the *in vivo* samples.

Results: Regarding the mechanical response in the *ex vivo* groups, the continuous suture had the highest failure membrane tension, and the methods using glue showed the lowest failure membrane tension values. However, the single interrupted and continuous suture had the highest failure stretch values. For the *in vivo* groups, the mechanical response values were similar between groups. However, the continuous suture had the highest stretch value, and the methods using glue showed the lowest stretch values. *in vivo*, all groups showed good tissue integration of the mesh regardless of the fixation method used. The histological study showed a more dense and mature repair tissue when the cyanoacrylate was used as a spray.

Conclusions: There are potential clinical advantages to the glue-spray method where viscosity effect and the polymerization time should be less important at the application time. At short term, the method of fixation is mechanically irrelevant as the mesh-tissue interface is exceedingly stronger than tissue or the mesh itself, however maturation of scar tissue was faster when spray was used.

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ACTIVE PROLIFERATION OF C-CELLS OF ULTIMOBRANCHIAL ORIGIN IN THE THYROID GLAND OF AN OLD RAT

Victoria Vázquez-Román[1], José C Utrilla[1], José Mª Fernández-Santos[1] e Inés Martín Lacave[1].

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

Introduction: Recently, it has been proved that the two endocrine cell populations that integrate the thyroid share an embryonic origin in the endoderm. While follicular cells come from the primitive pharyngeal floor, the C-cells come from the last pair of branchial pharyngeal pouches, which give rise to the ultimobranchial bodies (UBB). UBBs migrate until disperse in the thyroid lobules giving rise to C-cells during fetal life and persist as an embryonic remnant called *"solid cell nests"*, in humans, or UB follicles (UBF), in rodents, with an unknown function in postnatal life. Our research group has recently verified that UBFs retain undifferentiated cells in their walls able to give rise to both new C-cells and follicular cells. The present work shows a case of aberrant development of an UBF in which the ability to produce C-cells persists intensely during adulthood.

Material and Methods: We have analyzed the aberrant thyroid of a 15-month-old rat that was previously fixed in formaldehyde and paraffin-embedded. After serially sectioning the gland, immunohistochemical analysis was performed using the following primary antibodies: anti-Calcitonin, anti-Thyroglobulin, anti-Cytokeratins (34βE12), anti-p63 and anti-TTF-1. EnVision-Flex was used as developing system and diaminobenzidine. On several sections, PAS technique was also performed.

Results: Different cellular types could be detected in this anomalous UBF. Besides ciliated and mucinous cells constituting the apical part of the cyst epithelial wall, numerous undifferentiated cells immunopositive for p63 and CK-34βE12 were observed at peripheral level. Cells positive for TTF-1 and negative for undifferentiated markers were also identified. Nevertheless, the most outstanding finding was the elevated number of C-cells generating from the periphery of the UBF wall, which eventually emigrated to be incorporated in the surrounding thyroid tissue.

Conclusions: The persistence of an atypical UB remnant at thyroid level in an adult rat constitutes a paradigmatic example of the persistence of endoderm stem cells that could differentiate either in C cells or follicular cells in postnatal life.

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EFFECT OF HYPOXIA IN POST-HATCHING DEVELOPMENT OF SALMON Salmo salar SPINAL CORD.

Mariana Rojas[1], Hilda Hernández[1], Eugenia Díaz[1], Mariano del Sol[2].

[1] Program of Anatomy and Developmental Biology, ICBM, Faculty of Medicine, University of Chile.
[2] Doctoral Program in Morphological Sciences University of La Frontera, Temuco, Chile.

Introduction: Hypoxia has a teratogenic effect on the fish during embryonic development. Nevertheless, the effects on the larval stage are yet not known. The aim of this study was to asses the effects of hypoxia on the number of neurons and their apoptotic rate in the spinal cord of alevín.

Material and Methods: Salmo salar after hatching. A total of 400 alevines were used, establishing both hypoxia and control (normoxia) groups, considering the post-hatching days 1, 3, 5 and 7. Transversal sections were made of the whole alevin body at the level of the dorsal fin. Cresyl violet staining was carried out and the spinal neurons were counted by the method of optical dissector. Immunohistochemistry was performed against the transcription factor HIF-1α, and neuronal apoptosis was assessed by immunohistochemistry against Caspase 3. Statistical analysis ANOVA and Tukey's test were applied in order to assess significant differences between groups.

Results: Spinal cord of Salmon alevines is in a stage of morphogenesis topography, in concordance to that has been observed in other fish and mammals. HIF-1α was expressed in spinal neurons in both groups, being significantly higher in the hypoxic group. The process of neuronal apoptosis was observed in both hypoxic and normoxic groups, reaching higher significance in the hypoxic alevines. The number of neurons in the spinal cord was significantly lower in the hypoxic group. In conclusion, this work shows that hypoxia in Salmo salar farming fry posthatch decreases in half the number of neurons and increases the expression of HIF-1α and apoptosis in the spinal cord.

Conclusions: These results contribute to increase our knowledge about the biological development of salmon, in particular the genesis of the spinal cord, and the effects of hypoxic conditions on the development of this structure.

PACINIAN CORPUSCLES IN THE HUMAN PROSTATE

Marina Gándara-Cortés[1], José Antonio Ortiz-Rey[1], Débora Chantada[1], Laura Juaneda-Magdalena[1], Ángeles Peteiro[1], Pilar San Miguel[1], Joaquín González-Carreró[1].

[1]Department of Pathology, Álvaro Cunqueiro University Hospital, Vigo, Spain

Introduction: Pacinian corpuscles (PCs) are sensory mechanoreceptors predominantly found in the skin, which respond to rapid vibrations and deep mechanical pressure. They are usually found in the dermis and the subcutaneous tissue, with a wide distribution, specially in the fingers and toes, arms, neck, genitalia and nipples, but, even, in the mesentery, joints, periosteum, ligaments and tendons. There are isolated reports of PCs in some locations such as pancreas, urinary bladder, lymph node or prostate (only one case reported).
The finding of one case of PC in a prostatectomy in our department has led us to investigate the existence of this anatomical structure in a series of consecutive prostatectomy specimens.

Material and Methods: One hundred radical prostatectomy specimens consecutively received in our department were retrospectively reviewed searching specifically for the presence of PCs.
A total of 3.077 H-E slides from 100 specimens (14-85 per specimen, average= 30,77) were reviewed.
All of the PCs found were confirmed with immunohistochemical staining for EMA, which is expressed by the outer core and the capsule of the PCs.

Results: One PC was found in each one of 4 prostatectomy specimens (4% of our series of prostates). They were located in the peripheral fat tissue, at 402 to 1505 micra (mean: 960 micra) from the fibrous capsule. Three of them were in the right lobe (two of them in the lateral aspect of the mid region and one in the anterior base) and the other one in the left lobe (anterior aspect of mid region). The diameter of the PCs ranged from 358 to 829 micra (mean=694 micra).

Conclusions: The presence of PCs is a rare finding in prostate although not so unusual as described in the literature. A difference with the previously reported case (intraparenchimatous PC), our cases were located in the periprostatic fat tissue, although closely adjacent to the capsule. They were in both lobes and predominantly in the mid region, and lateral or anterior aspect. The morphology or size are similar to the PCs of the usual locations. The possible role of these PCs so close to the prostate or if this finding should be considered as an ectopia are issues that remain to be elucidated.

EFFECTS OF LOW-LEVEL LASER ON FIBROBLASTS OF HUMAN PERIODONTAL LIGAMENT: SYSTEMATIC REVIEW

Naira Figueiredo Deana [1], Nilton Alves [2,3], Paulo Sandoval [3]

[1] Master Program in Dentistry, Faculty of Dentistry, Universidad de La Frontera, Temuco, Chile.
[2] Applied Morphology Research Centre (CIMA), Faculty of Dentistry, Universidad de La Frontera, Temuco, Chile
[3] Faculty of Dentistry, Universidad de La Frontera, Temuco, Chile.

Introduction: Fibroblasts of periodontal ligament (PDL) play a key part in fixing the tooth in the alveolar bone and in maintaining homeostasis of the surrounding tissues, such as the alveolar bone and the cementum. A temporary inflammatory process is triggered in various clinical situations, determining the synthesis of prostaglandins and cytokines in fibroblast cells derived from human PDL. As low-level laser (LLL) has bio-stimulant, anti-inflammatory and analgesic effects, the object of this study was to carry out a systematic review of the effect of LLL on cultures of human PDL cells.

Material and method: A search was made of the Pubmed, WoS and SCOPUS databases using search terms "low-level laser", "periodontal ligament cell", "periodontal ligament fibroblast". To select the studies, first the relevance of the titles and abstracts was analysed, then studies were selected according to the inclusion/exclusion criteria. Studies were included which analysed cell proliferation, cytokine release and protein synthesis with red or nearinfrared lasers. Systematic reviews, studies in animals, studies of other types of fibroblast, highlaser and infrared laser were all excluded.

Results: 106 studies were identified. After exclusion of duplicate studies, 62 studies were excluded as irrelevant and 24 studies were selected for analysis. After application of the inclusion/exclusion criteria, 9 studies were selected for the review. These studies reported the use of InGaAlP 660 nm and GaAlAs continuous mode 670-830 nm laser equipment, with power from 10 to 500 mW. It was observed that the laser-irradiated cell cultures showed higher activity than the controls. Doses from 1 J/cm2 effectively increased cell proliferation. PGE2 inhibition was observed with a dose of 3.82 J/cm2. Doses of 5-10 J/cm2 were used to reduce the inflammatory marker expression (iNOS, TNF-α, and IL-1, COX-2).

Conclusion: All the studies reported that irradiated cell cultures obtained better results than the controls. LLL is capable of increasing human PDL cell proliferation and decreasing cellular inflammatory marker expression; the increase in PGE2 production is significantly inhibited by laser irradiation in a dose-dependent manner.

MORPHOMETRIC EFFECTS CAUSED BY INFRARED LASER OVER THYROID TISSUE

Ricardo Cornejo [1]

[1] *Facultad de Medicina, Universidad de La Frontera, Chile.*

Introduction. The thyroid gland have a great important due to the synthesis and secretion of hormones involved in the animals physiology. Due to its importance the aim of the present study was to determine evaluations of thyroid structure submitted to infrared laser stimulated.

Material and Method. Ten Sprague Dawley rats of three months old, weighing approximately 200 grams each, were divided into two groups of 4 animals each: the group control and the 4 animals in the experimental group received infrared laser stimulation to the thyroid with doses of 16 J/cm2 for 15 consecutive days. Once sacrificed, the respective thyroids were extracted to be processed for optical microscopy and histological and micrograph slides were made with final increases of up to 1000 X. Morphometric studies were conducted on 40 histological slides to determine tissue variations generated by the infrared inductions, with special emphasis in the colloidal disposition, constancy of blood vessels and dimensions of thyroid follicles.

Results. The results show that colloidal area, the blood vessels area and the thyroid follicles measurements decreased.

Conclusions. The inductions of the infrared laser compared to the controls revealed noticeable differences in all the components of the thyroid tissue analyzed, which could provide background on different functionalities in the metabolism of the respective glands.

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EFFECTS OF DIFFERENT FIXATIVES ON HISTOMORPHOLOGY OF THE RETINA OF TELEOSTS AND MAMMALS

Rosa Álvarez-Otero[1], Ainhoa Rodríguez-Tébar[2], Encarnación de Miguel[1,2]

[1] Departament of Functional Biology and Health Science, University of Vigo, Spain.
[2] CINBIO. Centro Singular de Investigación de Galicia 2016-2019, University of Vigo, Spain.

Introduction: Preservation of tissues is a critical part of the histological techniques. A wide variety of fixative liquids have shown to be excellent choices to successful histological research of mammalian tissues. However, aquatic animals may require the use of particular fixative solutions.

Material and Methods: We have evaluated the incidence of different fixatives (Bouin, Carnoy, modified Davidson's fluid)-, neutral buffered formalin, and 4% paraformaldehyde in 0.1M phosphate buffer) on the morphology and quality of the stain in the eye of teleosts (*Oncorhynchus mykiss*) and mammals (Swiss CD-1 mice). After anesthesia, animals were killed and the eyes were enucleated, fixed for 24 hours, processed according to a routine paraffin protocol and stained with hematoxylin-eosin.

Results: The retina of both mouse and trout have a laminated organization. As a general rule, all fixatives used yield an adequate preservation of the retina of mice, although the clearest stain is observed in retinas fixed by Davidson's fluid. In trout, retinal histology is significantly well preserved when Davidson's fluid and paraformaldehyde was used. Also in trout, Davidson's fixative produce the most defined stain pattern. By contrast, the remainder fixatives yield shrinkage artefacts which mainly affect to the inner nuclear layer. While the shrinkage grade is weak in retina fixed by Bouin and Carnoy, it was particularly evident in the eyes fixed by neutral buffered formalin.

Conclusions: In summary, the nature of the fixative has a major influence on the preservation of the retina of trout, especially in the inner nuclear layer that collapse significantly when neutral buffered formalin is used. A minor influence of fixatives is observed in the retina of mice.

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OPTIMIZATION OF IMMUNODETECTION OF GLUCOSENSING MARKERS IN RAINBOW TROUT TISSUES

Rosa Álvarez-Otero, Cristina Otero-Rodiño, Cristina Velasco, Rosa Ceinos, Marta Conde-Sieira, Sara Comesaña, José Luis Soengas.

Department of Functional Biology and Health Science and ECIMAT-Galician Singular Marine Research Centre, University of Vigo, Vigo (Pontevedra), Spain.

Introduction: One of the difficulties of immunohistochemical techniques (IHC) is to preserve tissue morphology and antigenicity. In this way, it is accepted that aldehyde-fixed and paraffin-embedded tissue sections need to undergo antigen retrieval (AR) before immunohistochemical procedure. However, there is no standard processing method common to all tissues and antibodies, so the optimal conditions must be determined by the user for each experiment.

Material and Methods: In this study, we assessed the optimization of the immunostaining of three proteins -glucokinase-independent glucosensing markers- in different tissues of the rainbow trout: 1) sodium/glucose co-transporter 1 (SGLT-1) in kidney, gut, and brain. 2) a nuclear receptor that regulate transcription of genes for cholesterol metabolism, cholesterol transport, and lipogenesis (liver X receptor alpha, LXRα) in liver and brain. 3) a G-protein coupled receptor involved in taste responses (sweet taste receptor, TAS1R3) in brain. Three fixatives were tested: Bouin, neutral buffered formalin and 4% paraformaldehyde in 0.1M phosphate-buffered saline (PBS, pH 7.4).

Results: Evaluation of IHC-stained slides indicated that in all tissues assessed, neutral buffered formalin and 4% paraformaldehyde provided the best results regarding tissue morphology and immunostaining. AR was performed in sections from paraffin-embedded tissue before immunostaining. The target antigens were retrieved by microwaving the sections in either Tris–ethylenediaminetetraacetic acid (EDTA) buffer (pH 9.0) or sodium citrate buffer (pH 6.0). The samples were heated in microwave at 700 W for 5 or 10 min after to initiate boiling of the solution.

Conclusions: Heat-induced AR is necessary for LXR and TAS1R3·in Tris-EDTA buffer (pH 9.0) both in liver as in brain. We obtained good immunostaining without AR in gut and kidney but AR procedure in citrate buffer (pH 6.0) is necessary in brain. No differences were observed between two times of boiling used.

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IMPROVING MANAGEMENT AT DIAGNOSIS IN AN ANIMAL MODEL OF CROHN'S DISEASE

Tamara Ortiz[1], Matilde Illanes[1], Josefa-María García-Montes[2], Enrique Melguizo Madrid[3], Francisco Luís Bellido[4], Federico Arguelles-Arias[4], Manuel De-Miguel[1]

[1] Dpto. Citología e Histología Normal y Patológica, Facultad de Medicina, Universidad de Sevilla
[2] Dpto. Medicina, Facultad de Medicina, Universidad de Sevilla
[3] Dpto. Bioquímica Clínica, Hospital Universitario Virgen Macarena, Sevilla
[4] Servicio de Aparato Digestivo, Hospital Universitario Virgen Macarena, Sevilla

Introduction: Crohn's disease (CD) is an inflammatory bowel disease (IBD), whose pathogenesis and etiology remains unclear. Pathological alterations associated include intestinal inflammation, alteration of the intestinal epithelial barrier and formation of ulcers. Our objective was to simulate the pathogenesis and clinical characteristics of CD and to validate a more efficient histopathological evaluation methodology.

Material and Methods: Male Balb/c mice (25-28 g) of 8 month old were obtained. For the induction of the disease we used trinitrobenzenesulfonic acid (TNBS), a heptane that, together with ethanol, acts as a disruptor of the intestinal barrier. TNBS was prepared at a concentration of 3 mg with 50% etanol in a total volume of 70 µl and was administered via intracolonic. Mice control group received the same volume with only 50% ethanol.

Results: Mice were sacrificed in the presence of clinical signs by intraperitoneal anesthesia. The large intestine was removed and opened by longitudinal incision. Macroscopic assessment of the intestine was performed, in which the control mice showed hyperemia and thickening in the proximal colon without ulceration, whereas the intestine in the presence of TNBS also presented congestion in the distal part. Subsequently, the longitudinal fraction was rolled from the distal to the proximal end in order to evaluate the tissue and its characteristics in only one slide. Histopathological study of the samples revealed loss of mucous secretion at the focal level together with inflammatory infiltrate of neutrophil predominance in the lamina propria, whereas in some glands it was possible to observe few neutrophils, manifesting a process of acute inflammation. Adipose tissue showed few foci of lymphocytic infiltrate. No granulomas were present.

Conclusions: Although this animal model does not reproduce the etiopathogenesis of CD, it does allow us to understand aspects related to the appearance of periods of the active inflammation and the clinical course of the disease; in addition to providing us with the possibility of developing new therapeutic strategies. The methodology used provides a different and easy approach to perform studies of Crohn's disease through the evaluation of the entire length of the large intestine coiled in the same slide.

ANALYSIS OF HISTOLOGICAL PATTERNS OF HUMAN PALMOPLANTAR SKIN EPIDERMIS

Vela-Romera A[1], Prados-Olleta N[2], Martín-Piedra MA[1], Jaimes-Parra BD[1], Campos F[1], Carriel V[1], Alaminos M[1], Garzón I[1]

[1] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[2] Division of Trauma and Orthopedic Surgery, University Hospital Complex of Granada and Department of Surgery, University of Granada, Spain

Introduction: The skin structure and function vary according to the anatomical area in which it is found. Two types of skin can be found according to its thickness: the thick skin found in feet soles and hand palms, and the thin skin found at most of the body surface. Despite its functional relevance, the histological structure of the palmoplantar thick skin has not been well characterized to the date. The objective of this work is to perform a characterization of the skin epidermis of the palmoplantar region to determine its histological patterns as compared to human thin skin epidermis.

Material and methods: For this study, thick and thin human skin biopsies were obtained from the same donors, fixed in formalin and methacarn and embedded in paraffin. For histological analysis of the epidermal layer, tissue sections were stained with hematoxylin-eosin and Fontana-Mason-Picrosirius. To evaluate the presence of reticular fibers at the basement membrane, Gomori's reticulin histochemical methods were used. Identification of melanocytes and Langerhans cells was carried out by MELAN-A and CD1A immunohistochemistry. The number and size of cells of the epithelial layer were determined in each type of skin by using ImageJ software.

Results: Our results demonstrate that the higher thickness of the palmoplantar skin epidermis is a consequence of a higher number and size of the epidermal keratinocytes. In addition, finger-like structures were found at the basal stratum of palmoplantar skin epithelium. Analysis of other cell types at the epithelial layer revealed a lower number and size of melanocytes and Langerhans cells for the thick skin. At the basement membrane level, abundant reticular fibers were found in the palmoplantar skin as compared to thin skin.

Conclusions: Our results suggest that thick skin epidermis is associated to an increased number and size of epithelial keratinocytes and lower melanocytes and Langerhans cells, and this epithelium is attached to the subjacent dermis by a thicker basement membrane. The study of the histological patterns of palmoplantar skin may lead to the better understanding of pathologies affecting palmoplantar skin areas and could contribute to improved treatment strategies.

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DIFFUSE BRAIN INJURY MARMAROU RODENT MODEL. UPDATED AND CRITICAL REVIEW

Alberto Fernández Liste[1,2], Antonio González-Cantalapiedra[3], Rosalía Gallego[1], Tomás García-Caballero[1], José Luis Cascallana[2]

[1]Departamento de Ciencias Morfolóxicas, Facultade de Medicina, Universidade de Santiago de Compostela
[2]Instituto de Medicina Legal de Galicia (Imelga). Xunta de Galicia
[3]Departamento de Anatomía, Producción animal y ciencias clínicas veterinarias, Facultade de Veterinaria de Lugo, Universidade de Santiago de Compostela

Introduction. A new model of diffuse brain injury in rats was published by Marmarou and Foda many years ago (1996). They described a method to produce brain acceleration throw a head impact. The injury was induced via the release of 450 g from a height of 1 or 2 m onto the intact skull which was protected by a steel disc to prevent skull fracture. The head was free to move on a 'foam bed' or cushion providing the translational acceleration and deceleration components of the injury. The objective was to identify the trauma levels that would induce mild head injury (with no mortality) and severe head injury (about 50% mortality rate) with a low incidence of skull fracture. From that moment on, many authors have been using this method achieving really different results by implementing the same parameters.

Material and methods. In our field of research we needed to inflict the most severe closed head injury making sure that the rats survive at least for 24 h in order to test specific cerebral protein expression. Following Marmarou model, we started releasing 450 g weight from 2 m high, but most of the rats died immediately. So we tried with different heights going down to 1 m high.

Results. We got some good positive protein-tissue expression but the immediately rat mortality was 50%, and 25% of the surviving rats showed minimal injuries. The explanation lies on the extremely steep injury tolerance curve of the rat skull. Beside, very subtle changes during the impact, such as movement of the head due to respiration and others, might dramatically change the intensity of the injury. We also altered the steel disc making it wider. The foam density used to lay the rat on is the other factor. According to the degree of density, the head of the rat moves down more or less after the impact, modifying consequently the results.

Conclusion. Little changes in the point of impact and the density of the foam are able to alter heavily the results, so they are two essential factors to consider in standardisation of Marmarou rodent model.

THE NKB/DYN-KISS-GNRH SYSTEM IN NON-CANONICAL HYPOTHALAMIC NUCLEI OF SOUTH AMERICAN PLAINS VIZCACHA, *LAGOSTOMUS MAXIMUS*, (RODENTIA, CAVIOMORPHA).

Schmidt AR[1,2], Inserra PIF[1,2], Charif SE[1,2], Cortasa SA[1,2], Proietto S[1,2], Corso MC[1,2], Halperin J[1,2], Vitullo AD[1,2], Dorfman VB[1,2].

[1]Laboratorio de Neuroendocrinología de la Reproducción, Centro de Estudios Biomédicos, Biotecnológicos, Ambientales y Diagnóstico (CEBBAD), Universidad Maimónides, Argentina.
[2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

Introduction: Vizcacha, a rodent that inhabits the Pampean region of Argentina, shows pre-ovulatory follicle formation during pregnancy with an ovulatory process around mid-gestation. We have described the localization of the gonadotropin-releasing hormone (GnRH) in neurons of the preoptic (POA) and supraoptic (SON), and in axonic varicosities of arcuate nucleus (ARC) and median eminence (ME) of female vizcachas. In addition, estrogen receptor alpha (ERα) and progesterone receptor (PR) were localized in GnRH neurons of POA and SON. In other species, GnRH is modulated by factors as Kisspeptin (Kiss), Neurokinin-B (NKB) and Dynorphin-A (Dyn). Kiss conveys feedback of steroid hormones on GnRH modulation, while NKB and Dyn colocalize with Kiss neurons in ARC (KNDy cells) modulating them. KNDy cells also show ERα and PR expression and direct inputs to GnRH neurons of POA and ARC. The aim was to describe KNDy cells in the hypothalamus of the vizcacha and its relation with GnRH and hormone receptors involved in the regulation of the reproduction.

Material and Methods: We used female vizcachas (n=7) to identify GnRH, Kiss, Kiss receptor (GPR54), NKB, DYN, ERα, and PR by immunofluorescence and confocal microscopy. Nissl staining was performed to localize hypothalamic nuclei.

Results: GPR54 colocalized with GnRH neurons in the POA and SON, and Kiss localized in cytoplasms of neurons of POA and in fibers of SON and ARC. NKB and Dyn colocalized in somas and fibers of POA and SON, and in a few neurons and fibers of ARC. However, Kiss was not detected in NKB/Dyn neurons. In addition, ERα and PR colocalized in somas of either Kiss or NKB/Dyn cells.

Conclusions: This is the first description of the localization of members of the NKB/Dyn–Kiss-GnRH system in the hypothalamus of the vizcacha. The presence of the NKB/Dyn–Kiss system in different POA neurons, and the lack of Kiss in ARC, suggest an indirect model of NKB/Dyn–Kiss system action on GnRH regulation. Present results may indicate that in NKB/Dyn-Kiss-GnRH system, GnRH neurons would be influenced by the hormonal environment via ERα and PR, and indirectly by Kiss and NKB/Dyn.

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HYPOTHERMIA PREVENTS RETINAL DAMAGE GENERATED BY OPTIC NERVE TRAUMA IN THE RAT

Manuel Rey-Funes[1], Ignacio M. Larrayoz[2], Daniela S. Contartese[1], Manuel Soliño[1], Anibal Sarotto[1], Martín Bustelo[3], Martín Bruno[3], Verónica B. Dorfman[4], César F. Loidl[1], Alfredo Martínez[2]

[1]Laboratorio de Neuropatología Experimental, Instituto de Biología Celular y Neurociencia "Prof. E. De Robertis" (IBCN), Facultad de Medicina, Universidad de Buenos Aires, CONICET, Buenos Aires, Argentina.
[2]Angiogenesis Study Group, Center for Biomedical Research of La Rioja (CIBIR), Logroño, Spain.
[3]Laboratorio de Neurociencias, Facultad de Ciencias Médicas, Universidad Católica de Cuyo, San Juan, Argentina.
[4]Centro de Estudios Biomédicos, Biotecnológicos, Ambientales y Diagnóstico (CEBBAD), Universidad Maimónides, Buenos Aires, Argentina.

Introduction: Ocular and periocular traumatisms may result in loss of vision. Hypothermia provides a beneficial intervention for brain and heart conditions and, here, we study whether hypothermia can prevent retinal damage caused by traumatic neuropathy.

Material and Methods: Intraorbital optic nerve crush (IONC) or sham manipulation was applied to male rats. Some animals were subjected to hypothermia (8ºC) for 3 h following surgery. Thirty days later, animals were subjected to electroretinography and behavioral tests.

Results: IONC treatment resulted in amplitude reduction of the b-wave and oscillatory potentials of the electroretinogram, whereas the hypothermic treatment significantly ($p<0.05$) reversed this process. Using a descending method of limits in a two-choice visual task apparatus, we demonstrated that hypothermia significantly ($p<0.001$) preserved visual acuity. Furthermore, IONC-treated rats had a lower ($p<0.0001$) number of retinal ganglion cells and a higher ($p<0.0001$) number of TUNEL-positive cells than sham-operated controls. These numbers were significantly ($p<0.0001$) corrected by hypothermic treatment. There was a significant ($p<0.001$) increase of RNA-binding motif protein 3 (RBM3) and of BCL2 ($p<0.01$) mRNA expression in the eyes exposed to hypothermia.

Conclusions: Hypothermia constitutes an efficacious treatment for traumatic vision impairing conditions, and the cold-shock protein pathway may be involved in mediating the beneficial effects shown in the retina.

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CELL PROLIFERATION AND NEUROGENESIS WITHIN THE SUBVENTRICULAR ZONE OF THE NEONATAL PIGLET ARE MAINTAINED BY REMOTE ISCHEMIC POSTCONDITIONING

Daniel Alonso-Alconada[1,2], Pierre Gressens[3,4,5,6], Nicola J Robertson[1].

[1]Institute for Women's Health, University College London, London, UK.
[2]Department of Cell Biology and Histology, School of Medicine and Nursing, University of the Basque Country, Leioa, Bizkaia, Spain.
[3]Department of Perinatal Imaging and Health, Division of Imaging Sciences and Biomedical Engineering, King's College London, King's Health Partners, St. Thomas' Hospital, London, UK.
[4]Inserm, U1141, Paris, France.
[5]University Paris Diderot, Sorbonne Paris Cite´, Paris, France.
[6]PremUP, Paris, France.

Introduction: The majority of the 1.15 million babies developing neonatal encephalopathy each year occur in low and middle income settings, where therapeutic hypothermia is not available or safe. Remote ischemic postconditioning (RIPostC), a simple, cost-effective and safe strategy, has recently shown to be protective after global cerebral hypoxic-ischemic (HI) injury in newborn piglets. To evaluate the neurogenic response after hypoxia-ischemia (HI) and RIPostC in the subventricular zone using a piglet model of neonatal encephalopathy.

Material and methods: 22 newborn piglets were randomised to: (i) Naive (n=6): piglets with no insult; (ii) HI (n=8): No intervention after quantified transient HI insult; (iii) HI+RIPostC (n=8): four 10 minute cycles of bilateral lower limb ischaemia/reperfusion started immediately after hypoxia-ischaemia. At 48 hours after HI, neuroblast chains around SVZ were evaluated by H&E staining and Doublecortin (DCX) immunohistochemical analysis. Cell proliferation and neural stem/progenitor cell proportion were calculated by Ki67 and Ki67+Sox2 positive cell quantification, respectively.

Results: The significant decrease ($p<0.01$) in the area of neuroblasts chains (hematoxylin staining) and migrating neuroblasts (DCX) induced by HI was reverted with RIPostC, showing similar values to those of Naive animals. In the same way, RIPostC also obtained higher counts ($p<0.01$) for Ki67 cell proliferation marker and Ki67/Sox2 positive cells when compared to HI group.

Conclusions: Using a piglet asphyxia model, hypoxic-ischemic-induced reduction in both cell proliferation and number of neural precursor was ameliorated by RIPostC, returning to naïve levels.

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RETINAL HISTOGENESIS IN THE ZEBRA FINCH (*Taeniopygia guttata*): A MORPHOLOGICAL STUDY

Gervasio Martín-Partido[1], Guadalupe Álvarez-Hernán[1], Elena Sánchez-Resino[1]; Ismael Hernández-Núñez[1]; Alfonso Marzal[1]; Joaquín Rodríguez-León[2], Javier Francisco-Morcillo[1].

[1]Departamento de Anatomía, Biología Celular y Zoología, Facultad de Ciencias, Universidad de Extremadura, 06006 Badajoz, Spain.
[2]Departamento de Anatomía, Biología Celular y Zoología, Facultad de Medicina, Universidad de Extremadura, 06006 Badajoz, Spain.

Introduction: Among birds, the developmental rate and acquisition of retina structures is highly variable. Several characters are typically associated with altricial or precocial life-history styles. To date, most avian embryological studies have been developed in precocial species, such as the chicken and the quail. The zebra finch (*Taeniopygia guttata,* Vieillot 1817) is an altricial songbird belonging to the large avian order Passeriformes that has particular impact in neurobiology and developmental biology. The young zebra finches hatch blind, naked, and with less-well developed organs of locomotion.

Material and Methods: In the present study we have used zebra finch embryos ranged from stage 9 (St9) to St45 (Murray et al., 2013; J Morphol, 274:1090-1110) in order to detail the major events of retinal histogenesis. Specimens were fixed in 4% paraformaldehyde in phosphate buffer and embedded in Spurr's resin. Serial 2 µm sections were cut in a Reichert-Jung microtome, stained with 1% toluidine blue, and observed using a Nikon Eclipse 80*i* microscope.

Results: Maturational events described in the present study were first detected in the central retina and, as development progressed, they spread to the rest of the retina following a central-to-peripheral gradient. The primary optic vesicles are visible at St9. The first optic axons were observed in the most-vitreal part of the retina at St25. We have found abundant pyknotic nuclei in the presumptive neural retina at optic cup stages and in the differentiated retina at perinatal stages. Furthermore, abundant ectopic cell divisions were found in the developing retina between St32-St38. The emergence of the inner plexiform layer starts at St38. The OPL evolves later, at St39.

Conclusions: While the total incubation time for the chicken is substantially longer than the incubation time for zebra finch (21 days vs 14 days), the chicken retina matures in less time than the zebra finch retina. The six last stages of chicken development are characterized primarily by growth, whereas in the zebra finch, maturation of different systems is already present by these stages.

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EXPRESSION OF CALRETININ AND PARVALBUMIN TRANSCRIPTS IN RETINAL CELLS IN DEVELOPING VERTEBRATE RETINA: A COMPARATIVE STUDY

Gervasio Martín-Partido[1], Teresa Pavón-Muñoz[1], Guadalupe Álvarez-Hernán[1], Abdelmalik Ayad[2], José Luis Ferrán[2], Luis Puelles[2], Joaquín Rodríguez-León[3], Javier Francisco-Morcillo[1].

[1]Departamento de Anatomía, Biología Celular y Zoología, Facultad de Ciencias, Universidad de Extremadura, 06071 Badajoz, Spain.
[2]Departamento de Anatomía Humana y Psicobiología, Facultad de Medicina, Universidad de Murcia, 30071 Murcia, Spain.
[3]Departamento de Anatomía, Biología Celular y Zoología, Facultad de Medicina, Universidad de Extremadura, 06071 Badajoz, Spain.

Introduction: Calcium binding proteins (CBPs) play important roles in neuronal function regulating neuronal development, growth and survival. The expression of CBPs has been used to characterize retinal populations of neurons in the vertebrate retina. Most of the studies showing CBPs expression in the visual system were carried by immunohistochemistry (IHC). In this work, we have studied the expression patterns of calretinin (CR) and parvalbumin (PV) transcripts and proteins in the developing visual system of chick and mouse using *in situ* hybridization (ISH) and IHC, respectively.

Material and Methods: ISH and IHC techniques were performed on retinal cryosections of chick and mouse embryos and post-natal specimens of mouse. The sections were observed using a Nikon Eclipse 80*i* microscope, and photographed with a digital camera (Axiocam HRc).

Results: *Cr* mRNA expression was detected at E4 in the ganglion cell layer (GCL) chick retina, whereas the onset of CR immunoreactivity was delayed until E5. As development proceeded, both the *Cr* transcripts and protein were observed in ganglion, amacrine, bipolar, and horizontal cells. Concerning to *Pv* mRNA, it was firstly detected in the inner nuclear layer (INL) at E12. However, PV immunoreactivity was found for the first time at E13. The expression patterns of *Pv* transcripts and PV protein are highly coincident, restricted to cells located in the vitreal region of the INL. Furthermore, scarce cells were also detected in the GCL with antibodies against PV, while no signal was found in this layer using ISH. In the mouse retina, *Cr* transcripts were already at E16.5 in the vitreal-most region of the neuroblastic layer detected, while CR immunoreactivity was found in the same region for the first time at E18. *Cr* transcripts and protein were restricted to ganglion and amacrine cells.

Conclusions: The present findings proved that mRNA ISH is comparable to IHC for evaluating the expression of CBPs in the developing visual system of vertebrates. However, *Cr* and *Pv* transcripts were detected earlier in development than CR and PV proteins.

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HISTOLOGICAL EVIDENCE FOR TERMINAL NERVE AND VOMERONASAL ORGAN CONTINUITY FOR PHEROMONE RESPONSE INTEGRATION IN WISTAR RATS.

Serrano, Hector2, Gaona-Domínguez, Saúl2, García-Suárez, Ma. Dolores1, Mendoza-Castillo, Mariana2, Casas-Ortega, Cinthya2, León-Angulo, Larisa G. 2, Gómez-Olivares José Luis2. Departamentos de 1Biología y 2Ciencias de la Salud. Universidad Autónoma Metropolitana Unidad Iztapalapa. San Rafael Atlixco 186, Ciudad de México, 09340, México.

Introduction: The vomeronasal organ (VNO) develops from the olfactory plate and is in close relationship and communication with GnRH+ hypothalamic neurons. Is responsible for detection of volatile pheromones that regulate social and sexual behavior. Once pheromones are detected by the VNO epithelial cells, they translate this stimulus into neuroelectric signals sent to different regions in the brain. Little is known about the physical association of the VNO neuroepithelia and those from the terminal nerve (TN). The goal of this communication is to give histological evidence of the VNO-TN for integration of behavioral response to pheromone stimulation.

Material and Methods: Six Wistar adult male and 6 adult's female Wistar rats were used. Animals were euthanized by lethal sodium pentobarbital overdose. VNO and TN were obtained from the upper palate of the skull and immediately fixed in buffered formalin for a 24 h period. Tissues were processed for paraffin inclusion and 5 µm sections were submitted to routine HE stains and Luxol fast blue-PAS-Haematoxylin (LPH) or Cajal stain. Histological preparations were analyzed with the Neurol plugin of the FIJI Image software.

Results: The histological analysis show the pseudostratified epithelia of the VNO distinguishable from the neuronal tissue of the TN. A highly and softly packaged neuronal tissue is in contact with secreted mucus that accumulates pheromones. There are rather few physical contact points between the VNO and TN although they have physical connections to other tissue structures like the hypothalamus for the VNO or the Masera organ by the TN. A physical continuity between the VNO and TN can be observed (figure 1) indicating the physical and physiological connection of the VNO-TN for pheromone-elicited stimulation.

Conclusions: The visual analysis of VNO and nerve fiber projections of TN indicate a constant communication between the neuroepithelium and the GnRH positive hypophysis center with the TN bypass that not only has the hypothalamus as destination but they also stimulate other behavior-associated region in the brain giving rise to a n specific pheromone response.

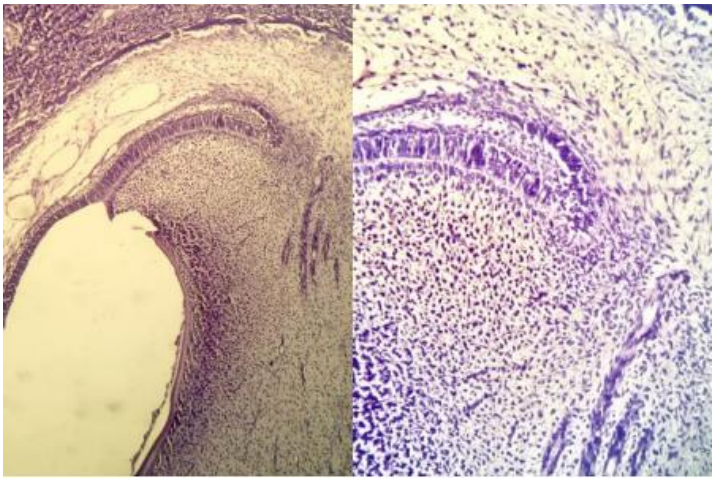

Figure 1. Histological characteristics of the Vomero Nasal Organ. The VMN pseudostratified epithelium can be clearly distinguished from the smooth tissue of the TN. VMNO tissue contains high amounts of neuronal cell bodies that are dispersed (left). Physical continuity between the VMNO neuroepithelia and TN tissue can
be observed (right)

THE NEURONS BEARING THE BASAL FOREBRAIN ARE CONNECTED WITH CORTICES OF DIFFERENT MODALITIES SHOWING SPECIFIC CONNECTING PATHWAYS DEPENDING ON THE NUCLEI OF STUDY.

Chaves-Coira I[1], Rodrigo-Angulo ML[1], Núñez A[1]

[1]Department of Anatomy, Histology and Neuroscience. Medical School. Universidad Autónoma de Madrid.

Introduction: The cerebral cortex receives consistent projections from cholinergic and non-cholinergic neurons bearing the basal forebrain (BF), which are implicated in attention, learning and memory processes and in the modulation of information processing. The goal of the present work is to determinate if the BF cortical projections are segregated in different neuronal populations for the different sensory modalities.

Material and Methods: The anatomical pathways linking BF neurons with somatosensory (S1), auditory (A1) and visual (V1) cortical areas were studied in 21 adult Sprage-Dowley rats by injecting the neuronal fluorescent retrograde tracers Fluoro-Gold (FG) in S1 and Fast Blue (FB) in either the visual-or-auditory cerebral primary cortices. After seven days animals were sacrificed and brains processed for visualizing the location of retrograde labelled neurons.

Results: Quantitative results of labelled neurons found in BF are referred in normalized percentages from total labelled ones. Animals receiving injections in S1 and A1 showed 98% FG-labelled neurons and 2% FB-labelled neurons in the Broca diagonal band (HDB); no double-labelled neurons were found in this region. In basal magnocellular nucleus (B), percentages of labelled neurons in these animals were 52% FG-labelled, 13% FB-labelled and 35% double-labelled neurons. Animals receiving injections in S1 and V1 showed 60% FG-labelled neurons, 12% FB-labelled neurons and 28% of double-labelled neurons in HDB. In B 39% of total labelled neurons were FG-labelled, 18% FB-labelled and 42% double-labelled neurons.

Conclusions: Our results suggest that the modulation performed by BF neurons on sensory cortices runs mainly throughout independent pathways. Since HDB display specific projections to cortices of different modalities, these neurons may play an important role in the integration and discrimination of the different sensory inputs.

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TRPC1 CHANNELS ARE EXPRESSED IN PYRAMIDAL NEURONS AND IN A SUBSET OF DENDRITE-TARGETING INTERNEURONS IN THE RAT NEOCORTEX

Juan R. Martinez-Galan, Ana Verdejo and Elena Caminos

School of Medicine/Institute for Research in Neurological Disabilities (Instituto de Investigación en Discapacidades Neurológicas-IDINE), Department of Medical Sciences, University of Castilla-La Mancha, Albacete, Spain.

Introduction: Canonical transient receptor potential (TRPC) channels are plasma membrane cation channels included in the transient receptor potential (TRP) superfamily. The most widely distributed member of the TRPC subfamily in the brain is TRPC1, frequently linked to group I metabotropic glutamate receptors (mGluRs) or to the components of store-operated calcium channels. TRPC activation could exert a neuroprotective effect during excitotoxicity, but a dysfunction lead to intracellular calcium disturbances and contributes to neurological disorders. The aim of our study was to analyze cellular and subcellular distribution of TRPC1 into the neocortex, to establish its function during different physiological and pathological events.

Material and Methods: Ten Wistar rats from P35 to P50 were used in double and triple-label immunofluorescence and confocal microscopy experiments to test the presence of TRPC1 in different layers, cell types and subcellular compartments, through combinations of TRPC1 with a series of specific neural markers.

Results: TRPC1 was abundant in SMI 32-positive pyramidal neurons, and in some GAD67 interneurons. On the contrary, TRPC1 was lacking in GFAP-positive glial cells. Regarding subcellular distribution, it was absent in synaptophysin-immunoreactive axonic terminals, but colocalized with postsynaptic marker MAP2 in cell bodies and apical dendritic trunks. TRPC1 totally overlapped group I mGluRs. Next experiments were to estimate the type of GABAergic interneurons where TRPC1 was present. There was no colocalization with parvalbumin, a marker of basket and chandelier interneurons, and only a low percentage of calretinin or calbindin interneurons expressed TRPC1. On the other hand, a 63% of somatostatin expressing-cells were positive for TRPC1. These double labeled cells were also positive for the glycoprotein reelin

Conclusions: 1) TRPC1 was mainly expressed in the soma and apical dendritic trunks of excitatory pyramidal neurons. 2) The presence of TRPC1 in reelin/somatostatin inhibitory interneurons points to the expression of this channel in a subset of dendrite-targeting interneurons, presumptively Martinotti and bitufted cells. Further experiments are needed to determine whether TRPC1 can be involved in some form of synaptic plasticity related to reelin pathway in adult neocortical interneurons.

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EFFECT OF CARNOSINE AND ALPHA-TOCOPHEROL ON CEREBRAL CORTEX IN MICE EXPOSED TO VANADIUM INHALATION. AN ULTRASTRUCTURAL ANALYSIS

L Colín-Barenque[1], CN Velázquez Pérez[2], D Altamira P[3]. Bizarro-Nevares[4], A. Gonzalez-Villalva[4], A. Zepeda[4] F.Pasos[4], P.Aley[1], J Espinosa-Villanueva[1], Fortoul T.I[4].
[1]Dept Neuroscience FES Iztacala, [2]Facultad de Ciencias, [3]FES Zaragoza, [4]Dept. de Biología Celular y Tisular Fac. de Medicina. UNAM. México

Introduction

Vanadium (V) is delivered to the atmosphere by the combustion of petroleum derivates rich in this element. V can induce the formation of reactive oxygen species (ROS) in biological systems and its toxicological effects have been associated with its production[1]. In previous studies we reported neuronal alterations in olfactory bulb and hippocampus in mice exposed to vanadium [2] Carnosine (beta-alanyl-l-histidine) and ∝-tocopherol have antioxidant activity as scavengers of free radicals[3]. The present study determined the effect of carnosine and ∝-tocopherol in mice prefrontal cortex exposed to vanadium by an ultrastructural approach.

Material and methods

CD-1 male mice weighing 30 ± 3g were divided into six groups. 1) Mice inhaled V_2O_5 (0.02M) one hour twice for a 4-week period. 2) V_2O_5 inhalation plus Carnosine oral treatment (1mg/kg/day) 3) Only Carnosine treatment, 4) V_2O_5 inhalation plus ∝-tocopherol (200mg/kg) IM twice a week 5) Only ∝-tocopherol and 6) Controls inhaled saline. Animals were sacrificed by pentobarbital overdose, perfused by intracardiac puncture with saline and glutaraldehyde (2%). Cerebral cortexes were dissected and processed for MET. The samples were analyzed in a Zeiss EM 100.

Results

The ultrastructural examination revealed that pyramidal neurons of cerebral cortex from carnosine group showed similar organelle ultrastructure -devoid of damage- as in controls. In contrast, alterations in pyramidal neurons were observed after V-exposure (dark cells with shrunken soma, pyknotic nucleus, a form of necrotic neuronal death). In particular, V-exposed mice and treated with carnosine showed a decrease in neuronal death compared with V-exposed without carnosine. In the ∝-tocopherol group some neurons with vacuolization were observed. In contrast, the pyramidal neurons of the group exposed to V and treated with ∝-tocopherol showed necrosis and ultrastructural alterations.

Conclusions

These results showed that ultrastructural alterations in pyramidal neurons death induced by V, is mediated by oxidative stress and that carnosine may modulate the neurotoxic vanadium action, unlike ∝-tocopherol that did not display any neuroprotective effect.

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CHARACTERIZATION OF A SPRAGUE DAWLEY RAT LINE BEARING A SPONTANEUS MUTATION: A NEW MODEL TO STUDY MYELINATION MECHANISMA AND DYSMYELINATING DISORDERS

Laura Martínez-Palma[1,4], Mariela Santos[2,4], Fernando Benavides[3], Sergio Rocha[2] , Karina Hernández[1], Martín Breijo[2].

[1]Departamento de Histología y Embriología, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay.
[2]Unidad de Reactivos y Biomodelos de Experimentación, Facultad de Medicina Universidad de la República, Montevideo, Uruguay.
[3]Department of Molecular Carcinogenesis, University of Texas MD Anderson Cancer Center, Texas, USA.
[4]equally contributed to the work

Introduction: Alterations of myelin formation and maintenance lead to neurological disorders due to axon conduction impairment. Here we describe a new mutant line of Sprague Dawley (SD) rats named *tembleque* (SDt) exhibiting tremor and myelin alterations.

Material and methods: Inheritance was studied by crossing male and female SDt with wild-type SD rats (F0) and intercrossing the F1 progeny. For histological and transmission electron microscopy (TEM) studies, animals were transcardially perfused with 4% paraformaldehyde or 3.5% glutaraldehyde, and brain, spinal cord and sciatic nerve were removed. Paraffin or cryostat sections were processed for hematoxylin and eosin staining or immunohistochemystry. Thoracic spinal cord and sciatic nerve were processed for semithin and ultrathin sectioning for TEM.

Results: Behaviour and inheritance characterization: SDt phenotype is characterized by tremors and abnormal locomotion involving the hind body and limbs, appearing by week 3 and decreasing gradually after week 16. Crosses between mutant SDt rats resulted in 100% SDt phenotype. F1 progeny from mutant SDt x SD crosses was 100% normal phenotype. Intercrossing F1 rendered around 25% of F2 with SDt phenotype. Morphological analysis: Sections from the spinal cord of young (5 weeks old) and adult (20 weeks old) showed reduced white matter area and more dense organization of axons and glial cells in SDt compared to SD animals. Immunohistochemical studies with myelin and glial markers are being performed. TEM studies showed important reduction in myelin sheets thickness and alterations in compaction. These findings were more evident in adult animals when symptoms have been reverted. Peripheral nerves did not show differences in SDt compared to SD animals.

Conclusions: The *tembleque* (SDt) rat develop a transient phenotype mainly characterized by hind body and limb tremors, consistent with an autosomal recessive mutation. Severe central nervous system hypo- and dismyelination was determined by the morphological analysis. SDt rat is a potential new model for studying the myelination process as well as the evaluation of new treatments directed towards diseases associated with myelin alterations.

Acknowledgements: To CSIC, UDELAR for financial support. To Gaby Martínez and Gabriela Casanova, from UMET, Facultad de Ciencias, UDELAR for help with TEM analysis.

STUDY OF THE CEREBELLUM AND BRAINSTEM OF RATS SUBMITTED TO AN EXPERIMENTAL MODEL OF GLOBAL ISCHEMIA BY CARDIAC ARREST

Hernández R, Pedrosa J.A, Peinado M.A, Blanco S.

Experimental Biology, University of Jaen

Introduction: Brain ischemia is among the main causes of mortality in Europe. Not all cerebral cells respond equally to this injury, i.e. neurons in the cerebellum and brainstem show different vulnerability to brain ischemia, whereas astrocytes play a neuroprotective role in the neuronal damage underlying ischemia. Among the complex metabolic reactions occurring in the process of ischemia, many are related to the formation of reactive oxygen species (ROS). Thus, the aim of this work is to carry out a comparative study of the parameters below in the cerebellum and brainstem of adult rats subjected to an experimental model of global ischemia by cardiac arrest (GICA).

Material and Methods: 1) The general state of the tissue by H-E staining, 2) location of glial fibrillary acidic protein (GFAP), as a recognized astrocyte reactivity mark, and 3) the determination of the levels of oxidative stress in lipids (detected by TBARS) generated as a central element of this differential vulnerability.

Results: The cerebellar cortex showed notable changes in the Purkinje cell layer by the effect of ischemia; thus, in the group of GICA animals, these neurons presented a high degree of degeneration, showing a hypertrophied soma and a hypochromatic nucleus. This microscopic results indicating tissue damage in the cerebellar cortex, were accompanied by an increase in GFAP expression, particularly in the molecular and granule cells layers; on the contrary, we have detected no significant changes in glial reactivity in the brainstem. Our results concerning TBARS, showed significant increases only after ischemia in the cerebellum but not in the brainstem.

Conclusions: This work shows that, following our experimental model, different cell populations within the brain, especially in the cerebellum, but not in the brainstem, are subjected to both morphological and molecular alterations. Our results show alterations of the cerebellum, mainly as a consequence of the production of ROS triggered by GICA. These findings open the door to new research that will allow to define the causes of differential susceptibility to cerebellar ischemia in detail.

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PROTECTIVE EFFECT OF EPIGALLOCATECHIN-3-GALLATE IN A MODEL OF CYRPTORCHIDISM AND SPONTANEOUS TESTICULAR DESCENT.

Estefania Reyes Cruz[1,2], Julio C. Rojas Castañeda[1], Margarita Chávez Saldaña[1], Francisco Jiménez Trejo[1],Rosa María Vigueras Villaseñor[1].

[1]Instituto Nacional de Pediatría; [2]Programa de Maestría en Ciencias de la Producción y Salud Animal-FMVZ-UNAM, Ciudad de México, México.

Introduction: Cryptorchidism (CO), has a high incidence in humans. However, this high incidence is found to decrease in the first six to twelve months of life, principally owing to spontaneous testicular descent. In this transient period, the testis is subjected to high temperature and as a result, an oxidative stress is generated. Such oxidative stress may affect the future survival and differentiation of the gonocytes to spermatogonia. Epigallocatechin-3-gallate (EGCG) has been shown to be an effective stabilizer of free radicals and efficient activator of antioxidant enzymes. Objective: To assess the protective effect of EGCG in a model of CO and spontaneous testicular descent.

Material and methods: Rats, aged three days postpartum (dpp) were distributed as follows: healthy controls (HC), HC with vehicles, HC with EGCG, transient CO induced by administration of 0.1 µg of 17β- estradiol from 3 to 33 dpp plus vehicles (CO/V), CO with 15 mg/kg of EGCG (CO/EGCG15) and CO with 25 mg/kg of EGCG (CO/EGCG25). At 86 dpp, the animals were euthanized and their histopathological alterations and the concentrations of testosterone were determined.

Results: In the HC groups, testicular descent was found to conclude at 21 dpp while in the group of CO, spontaneous descent finished at 43 dpp. In the HC group, the cytoarchitecture was found to be normal whereas the CO/V group showed cellular peeling, vacuolization and some tubules including detention of the spermatogenesis. However, in the CO/EGCG15 group, there was a significant reduction of the histological alterations along with an increase in tubular area without reaching the parameters observed in HC group. The administration of EGCG at 25 mg/kg portrayed higher damage than that observed in CO/V group.

Conclusions: These results suggest that treatment with 15 mg of EGCG allows complete spermatogenesis and favors fertility, probably by increasing the activity of endogenous antioxidant system and by scavenge free radicals generated due to high temperature the cryptorchid testes were subject to in their short period in this position.

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CHANGES OF LEYDIG CELL POPULATION IN SYRIAN HAMSTER DURING SPONTANEOUS RECRUDESCENCE AFTER TO A SHORT PHOTOPERIOD

E. Beltrán-Frutos, J. Martínez-Hernández, V. Seco-Rovira, C. Ferrer, L.M. Pastor

Department of Cell Biology and Histology, Aging Institute, IMIB-Arrixaca. School of Medicine, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, 30100 Murcia. Spain

Introduction: Syrian hamster (*Mesocricetus auratus*) is an animal of seasonal reproduction. When this animal is subjected to a short photoperiod his testes undergo a regression process that involves changes in the size and number of Leydig cells. During the recrudescence stage the testis regains its original size and shape as well as the population of these cells is restored. The aim of this work is to study the quantitative changes and proliferative activity of population of Leydig cells undergo during the recrudescence.

Material and Methods: We used a total of 23 Syrian hamsters (*Mesocricetus auratus*) distributed in 4 groups: control, Initial Recrudescence (IR), Advanced Recrudescence (AR) and Total Recrudescence (TR). The immunohistochemical technique was used to detect the presence of PCNA and to quantify its positivity.

Results: We observed positivity to PCNA in Leydig cells type A, in small cells near to the interstitial vasculature ("possible progenitors"), endothelial cells, pericytes and myoid cells. The total number of Leydig cells is lower in IR group than in the control group ($p < 0.05$). The number of Leydig cells of type B and C significantly lower in the recrudescence groups respect to control ($p < 0.05$). The total number of D cells (possible progenitor) significantly increased in IR and AR groups ($p < 0.05$). The percentage of Leydig cells type A positive for PCNA showed no significant differences during the recrudescence process. On the contrary percentage of D positive for PCNA decreased during recrudescence ($p<0.05$).

Conclusions: The proliferation of Leydig cells does not contribute significantly to restoration in number during the recrudescence, on the contrary, the differentiation of new Leydig cells from the progenitors and the reduction of Leydig cells necrosis probably restores the population of these cells during recrudescence.

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MORPHOLOGICAL AND ULTRASTRUCTURAL ANALYSIS OF LEYDIG CELL DURING THE SPONTANEOUS RECRUDESCENCE IN HAMSTER SIRIO.

E. Beltrán-Frutos, J. Martínez-Hernández, V. Seco-Rovira, C. Ferrer, L.M. Pastor

Department of Cell Biology and Histology, Aging Institute, IMIB-Arrixaca. School of Medicine, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, 30100 Murcia. Spain.

Introduction: Testicular interstitum of Mesocricetus auratus undergoes important histological changes during exposure to a short photoperiod with atrophy and a decrease in the number of Leydig cells. After the regression, in recrudescence, there is a restoration of the morphology and number of these cells which has not been studied in detail. The objective of our work is to perform a morphological and ultrastructural study of Leydig cells during restoration process after exposure to a short photoperiod.

Material and Methods: A total of 24 Syrian hamsters were divided into four groups: Control, Initial Recrudescence, Advanced Recrudescence and Total Recrudescence. The testes were processed for light microscopy and electron microscopy. H & E staining was performed.

Results: During the testicular recrudescence several cell types were identified in the testicular interstitium. With light microscopy we observed Leydig cells type A of different sizes in the intertubular space as well type B and C, the latter less frequently than in control animals. Near always to the vessels (perivascular compartment) we observed smaller cells, with rounded morphology and little amount of cytoplasm. Cells with spindle morphology in boundary tissue of seminiferous tubule wall (peritubular compartment) were also observed. Electron microscopy identified the same types of Leydig cells as light microscopy. Next to the vessels were identified small cells, with large nucleus and that are not surrounded by basement membrane. Also spindle cells with mesenchimal character were observed.

Conclusions: In conclusion, the presence of types A (with different sizes), B and C cells during the recrudescence reveals a heterogeneous population together with a diverse degree of cellular differentiation in the Leydig cells. The observation of cells in perivascular and peritubular compartments indicates the presence of possible progenitors that will differentiate in Leydig cells restoring the lost population during the regression. This study was supported by: 19892/ GERM/ 15 from F. Séneca, CARM.

STRUCTURAL AND MOLECULAR EFFECTS OF NEONATAL ANDROGENIZATION ON ADULT RAT UTERUS.

Gabriel Anesetti[1] and Rebeca Chávez Genaro[1].

[1]Department of Histology and Embryology, Faculty of Medicine, University of the Republic, Montevideo, Uruguay

Introduction: Exposure to androgens during early postnatal life is able to reprogram several female system inducing morphological, biochemical and/or functional alterations. Previously we have demonstrated the effects of neonatal androgens on cyst ovarian development, and their repercussions on ovary function. In this study we analyze the programming effects of neonatal testosterone (T) or dihydrotestosterone (DHT) on adult uterine tissues.

Materials and methods: Formalin-fixed uteri from Wistar rats (40, 90 and 180 postnatal days) neonatally exposed to testosterone, DHT or vehicle were paraffin-embedded, and sections (5 μm thick) were obtained. Morphometric analysis for uterine changes were performed using Hematoxylin-eosin and Cajal-Gallego trichrome stains. Immunohistochemical detection of androgen (AR) and estrogen-alpha (ERα) receptors as well as ki67 (a proliferation marker) were also performed. To estimate the expression of ERα or AR in different cell types as well as proliferative index, a quantitative image analysis was performed by analyzing the proportion of positive cells in several tissue compartments (luminal epithelium, glandular epithelium, stroma and muscular layer).

Results: In all treatmens, uterine weight increased with age. A significant increase in myometrial layer thickness was evident at PND180 in both androgenized groups. Only testosterone treated rats displayed a significant increase of epithelial thickness, characterized by a pseudostratified hyperplastic epithelium with less orderly cells and basally located nuclei. The number of endometrial glands decreased significantly in rats exposed to testosterone while it was intermediate in animals in the DHT group. No significant differences in proportion of ERα or AR immunostained cells were observed between groups. AR was absent from epithelial cells. The percentage of ki67-immunostained luminal epithelial cells was significantly decreased in testosterone group.

Conclusions: This work demonstrates that neonatal androgens induce long-term changes on the uterine tissues and that these are more severe when rats were exposed to an aromatizable androgen as testosterone.

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SEA URCHIN SPERM EXPRESS β2-ADRENERGIC RECEPTOR IN FLAGELLUM

Maria M Francou[1], Jose L. Girela[1], Alba De Juan[1], Jorge Ten[1,2], Rafael Bernabeu[2], Joaquín De Juan[1]

[1]Departament of Biotechnology, University of Alicante, Alicante, Spain.

[2]Instituto Bernabeu of Reproductive Medicine, Alicante, Spain.

Introduction. Adrenergic receptors are known to be G-protein coupled receptors, many of which have been shown to modulate the activity of membrane-associated adenylyl cyclases and their production of cAMP. The presence of adrenergic receptors was identified in mammalian sperm, but their functionality is still poorly understood. The non-mammalian models, like sea urchin (*Arbacia lixula*), have become of interest because they provide important data about the evolution of adrenergic receptors and their role in the reproduction. In this sense, β-adrenergic receptor blocker is found to induce polyspermy in sea urchin eggs. However, identification and topographical distribution of sea urchin β-adrenergic receptors are not known. Thus, the aim of this study was the identification and subcellular localization of the β2-adrenergic receptor in sea urchin sperm.

Material and Methods. Sperm samples were collected by intracoelomic injection of KCl solution. Sperm suspensions were fixed and prepared for confocal microscopy, field-emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM). The adrenergic receptor immunolocalization was performed with a primary antibody directed against β2, and a fluorescent-conjugated secondary antibody to confocal microscopy, or gold-conjugated secondary antibody to FESEM and TEM.

Results. Immunoreactivity study confirmed the presence of β2-adrenergic receptor in sea urchin sperm. Confocal analysis revealed a strong immunofluorescence labeling of the β2-adrenergic receptor in the flagellum. This adrenergic receptor distribution was confirmed through gold immunolabeling, which was analyzed by FESEM and TEM.

Conclusions: These data suggest that sea urchin sperm express β2-adrenergic receptor and their subcellular localization is confined to sperm flagellum.

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DISTRIBUTION OF MUCINS IN HUMAN ENDOCERVICAL GLANDS DURING THE PROLIFERATIVE PHASE

[1]Francisco García-Molina, [2]Vicente Seco-Rovira, [2]Jesús Martínez-Hernández, J., [2]Ester Beltrán-Frutos E., [2]Concepción Ferrer, [3] José María Rodríguez-Ingelmo, [3]María Encarnación Andrada-Becerra E., [2]Luis M. Pastor.

[1]Departamento Anatomía Patológica. Hospital Universitario Morales Meseguer. Murcia.
[2]Department of Cell Biology and Histology, Medical School, IMIB, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, Spain.
[3]Hospital General Universitario de Elche. Alicante.

Introduction: Cervical mucus changes during the menstrual cycle presenting different biophysical and biochemical characteristics, throughout the cycle. It is now accepted that cervical mucus is not a homogeneous but a heterogeneous entity that contains different types of mucus that vary in proportion throughout the cycle. It has been proposed that specific regions of the endocervical mucosa produce each of the mucus types but it is unknown whether their biochemical composition differs. This study uses conventional histochemical techniques for mucins to analyse secretion in the endocervical glands during the proliferative phase in order to determine whether there are differences between the different parts of the endocervical canal as regards the composition of sialomucins, sulfomucines and neutral mucins.

Material and Methods: For this purpose, three longitudinal sections were used: anterior, posterior and middle of the endocervical canal from the uterus of eight women presenting endometrium in proliferative phase. The study was carried out under the control of the ethical research committee and the Biobank of the General University Hospital of Elche. The sections were stained with Alcian Blue pH 2.5 and Alcian Blue pH 1 and Alcian Blue pH 2.5 -PAS. The canal was differentiated into three regions for analysis purposes: proximal, middle and distal (endocervix). In each of them, 20 fields were visualized with a 20X objective, and the intensity of the staining and the percentage of glands for each slide were evaluated. Subsequently, an average semiquantitative value for each preparation of the mucin content was calculated, combining both the percentage and intensity.

Results: The three endocervical zones presented abundant mucin secretion of a more acid than neutral character. The proximal and middle region especially showed sulfomucines and the distal region a mixture of sulfomucines and sialomucins.

Conclusions: During the proliferative or estrogenic phase, mucus with abundant sulphate or carboxyl groups exists throughout the endocervical canal. Their distribution is heterogeneous because the proportion of sialomucins increases with respect to sulfomucines from the proximal to the distal zone. This difference may be related to changes in the types of mucus and fluidity that occur during the estrogenic phase.

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STEREOLOGICAL ESTIMATES OF LENGTH AND SURFACE DENSITIES OF THE ACINI FROM NORMAL AND PATHOLOGICAL PROSTATE: GLOBAL AND LOCAL DIFFERENCES

Luis Santamaría[1], Laura Andrés-Delgado[1], Irene Chaves[1], Ildefonso Ingelmo[2], Fernando Teba[3].

[1]Department of Anatomy, Histology, and Neuroscience. School of Medicine, Autonomous University of Madrid, Madrid, Spain.
[2]Department of Anesthesiology. Hospital Ramón y Cajal, Madrid, Spain.
[3]Department of Surgery (Urology). Hospital de La Princesa, School of Medicine, Autonomous University of Madrid, Spain.

Introduction: The present study will apply stereological tools to find out if the dimensions of acini immunostained to cytokeratin 18 (ck18) show local changes in benign prostate hyperplasia (BPH) and prostate adenocarcinoma (Ca) compared to normal prostate (CTR), independently if global estimates were similar. Thus, the following parameters will be applied: acinar length density (L_V), and surface densities of acini for both luminal (S_V ep) and basal (S_V bas) compartments.

Material and Methods: Sections from CTR, BPH and Ca specimens were immunostained to ck18. Strips formed by adjacent quadrats were explored from all the groups. The result was a series of images, sized 512 x 7000 pixels. The images were processed using a stereological software. Strips were mounted from the images, using the Image J. Local measurements of L_V, S_V ep, and S_V bas were obtained: The strip was segmented in 50 columns orthogonal to the long axis. On each column, appropriate frames were superimposed for the estimation of both L_V and Sv. The results were drawn in a XY plot. Along the space series, local estimates were performed. The global measurements were averaged from local measurements. The results have been expressed as mean ± SEM. Local and global estimates were compared between CTR, BPH and Ca by ANOVA. ($p<0.05$).

Results: Global L_V was significantly increased in Ca and decreased in BPH. Global S_V ep and S_V bas were significantly increased in Ca, and not differences were detected between BPH and CTR. For local estimates, only in two points along the X-axis the L_V was significantly decreased in BPH respecting to CTR. Not local differences were observed for both S_V measurements between BPH and CTR. All parameters show in Ca significant increases in almost all local positions when comparing with the other groups.

Conclusions: 1-Both local and global measurements studied are suggesting remarkable differences between cancer and normal or hyperplastic benign conditions: the relative size of the glandular tree was increased in tumour growing.
2-The surface of stroma/epithelium interchange appears increased in the malignant lesions. This may be related to the ability of the tumor cells to spread.

SIALIC ACID RESIDUES REDISTRIBUTION AND TYROSINE PROTEIN PHOSPHORYLATION DURING HUMAN SPERM CAPACITATION AND ACROSOMAL REACTION

Gómez-Torres, María José[1,2]; Sáez-Espinosa, Paula[1]; Pérez-Rico, Elena[1]; López-Botella, Andrea[1]; Huerta-Retamal, Natalia[1]; Robles-Gómez, Laura[1]; Avilés, Manuel[3]; Romero, Alejandro[1]; Aizpurúa, Jon[4];

1 Departamento de Biotecnología, Universidad de Alicante, Alicante, España
2 Cátedra Human Fertility, Universidad de Alicante, Alicante, España
3 Departamento de Biología Celular e Histología, Universidad de Murcia e IMIB, Murcia, España.
4 IVF SPAIN, Reproductive Medicine, Alicante, España

Introduction: During spem capacitation tyrosine phosphorylation is strongly related to hyperactivation. Further, appropriate plasmatic membrane sialic acid location is necessary to ensure fertilization process. However, no data exist about concurrent analysis of both biomarkers in human spermatozoa. Due to that, the aim of this study was to characterize head sialic acid residues patterns considering the presence or absence of tyrosine phosphorylation at flagellum after spermatozoa *in vitro* capacitation (1h and 4h) and acrosomal reaction induction.

Material and Methods: Samples were obtained from normozoospermic donors and spermatozoa were capacitated by swim-up for 1h and 4h in a buffer containing BSA (5mg/ml). Acrosomal reaction was induced with calcium ionophore A23187 for 1h and sialic acid localization was evaluated by *Wheat germ* lectin conjugated to FITC. Tyrosine phosphorylation was evaluated using an anti-phosphotyrosine antibody produced in mouse and a secondary antibody against mouse IgG conjugated to Cy^3. A minimum of 100 cells per sample was observed by fluorescence and data were statistically analyzed by Student's t-distribution ($p < 0.05$).

Results: Regarding sialic acid location, results showed that in non-capacitated condition most of spermatozoa presented fluorescence at acrosomal region (Pattern 1) whereas after capacitation the most representative pattern was dotted fluorescence all over the head (Pattern 2). Moreover, no differences were found between different capacitation times and tyrosine phosphorylation.
After acrosomal reaction and using tyrosine phosphorylation as a second biomarker, it was observed that capacitated spermatozoa for 1h with phosphorylation showed a higher percentage of Pattern 2 compared to those in which phosphorylation was absent. However, these differences disappeared after 4h of capacitation, indicating reacted spermatozoa without tyrosine phosphorylation need more time of capacitation to redistribute sialic acid to this location.

Conclusions: Sialic acid redistribution after capacitation and acrosomal reaction suggesting a relevant implication in these processes. In addition, sialic acid location after acrosomal reaction is associated with flagellum tyrosine phosphorylation and with capacitation time. In conclusion, simultaneous analysis of highlighted biomarkers, such as tyrosine phosphorylation and sialic acid, allows a better understanding of spermatozoa molecular changes, providing a useful tool to identify heterogeneity and to improve selection methods.

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ACTIN AND CALICIN IMMUNOLOCALIZATION IN HUMAN SPERM VACUOLES

Gómez-Torres, Mª José [1,2], Luna-Romero, Javier[1,3], Avilés, Manuel[4]; Romero, Alejandro[1]; Aizpurua, Jon[2,5]

[1]Department of biotechnology, University of Alicante, Alicante, Spain
[2]Chair Human Fertility, University of Alicante, Alicante, Spain
[3]iGLS (Integrated Genetic Lab Services), Alicante, Spain
[4]Department of Cellular Biology and Histology, Faculty of Medicine, University of Murcia, Spain
[5]IVF Spain, Fertility clinic and assisted reproduction, Alicante, Spain

Introduction: Sperm nuclear vacuoles are subtle nuclear malformations subject to many studies and probably related to male infertility. However, their origin is unknown and is still debate topic. The vacuole formation has been related with failure of sperm chromatin condensation, DNA fragmentation, fertilization and implantation failures or miscarriages. Regarding nuclear vacuoles origin, acrosomal content through fluorescence microscopy and nuclear indentation packed with membranous material through optic microscopy with high magnification are described. Optic microscopic techniques enable us the observation of the external sperm's shape, but little is known about internal structure because assays with higher resolving power are required. In addition, the presence of some cytoskeletal proteins in sperm head was studied by immunofluorescence microscopy, but it is difficult to know the exact internal location of these proteins. Here, we report new data about the origin and nuclear vacuole content in human spermatozoa through a detailed ultrastructural morphology by using immunocytochemistry and transmission electron microscopy (TEM) techniques.

Material and Methods: Sperm samples were analysed from 2 normozoospermic subjects. Fresh samples were fixed and included in LR-White resin and ultrathin sections were taken. Immunocytochemistry was performed to determine the presence of cytoskeletal actin and calicin proteins. An anti-calicin IgG polyclonal antibody produced in goat and an anti-actin IgG polyclonal produced in rabbit were used as primary antibodies. To reveal its location, protein A conjugated to colloidal gold was used. The vacuole structure and morphology were evaluated by TEM.

Results: We identified actin and calicin in the sperm vacuoles. We also observed that the vacuoles are invaginations produced by nuclear envelope allowing us to determine that their content comes from the cytoplasm. Our findings show that the origin and content of the nuclear sperm vacuoles is cytoplasmic discarding their acrosomal or nuclear in origin.

Conclusions: The combined approach using immunocytochemistry and TEM analyses determine the actin and calicin presence in the sperm vacuoles and their genesis due to an invagination of the nuclear envelope. These data provide a new insight about vacuole origin and location in the human spermatozoa. Overall, our findings shed light on physiological function in motility, fertilizing ability or a relationship with sperm maturity.

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NEW METHOD TO SIMULTANEOUSLY CHARACTERIZE THE EXPRESSION AND IN SITU ACTIVITY OF ECTO-NUCLEOTIDASES IN HUMAN TISSUES

María Lina Villamonte[1], Benjamín Torrejón-Escribano[1,2], August Vidal[1,3,4], Jordi Ponce[3,5,] Xavier Matias-Guiu[3,4] and Mireia Martín-Satué[1,3]

[1]Departament de Patologia i Terapèutica Experimental, Facultat de Medicina i Ciències de la Salut, Campus Bellvitge, Universitat de Barcelona, Spain.
[2]Serveis Científics i Tecnològics, Campus Bellvitge, Universitat de Barcelona, Spain
[3]Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), Spain
[4]Servei d'Anatomia Patològica, Hospital de Bellvitge, Barcelona, Spain
[5]Servei de Ginecologia, Hospital de Bellvitge, Barcelona, Spain

Introduction: Extracellular nucleotides, such as ATP, and nucleosides, such as adenosine, act as autocrine and paracrine molecules that have multiple roles in virtually all organs and tissues, including female reproductive organs. Extracellular ATP and adenosine levels are regulated by the action of ecto-nucleotidases that hydrolyse ATP to adenosine. The aim of the present study was to set up a new method to simultaneously localize the cellular distribution and *in situ* activity of ecto-nucleotidases in tissue sections. We used this method to characterize the expression of ecto-nucleotidases in human oviducts.

Material and Methods: Cryosections of non-pathological human oviducts were obtained from salpingectomy at the Service of Gynecology of Bellvitge Hospital. Samples were incubated with the following primary antibodies against human enzymes: anti-nucleoside triphosphate diphosphohydrolase 1 (NTPDase1/CD39), anti-NTPDase2, and anti-placental alkaline phosphatase (PLAP). *In situ* activity reactions were performed on the same slides using the Wachstein/Meisel lead phosphate method with ATP or ADP as substrate. For alkaline phosphatase activity, the BCIP/NBT revealing reagent was used. The sections were then incubated with the appropriate Alexa Fluor-conjugated secondary antibodies and mounted with Prolong Gold antifade with DAPI medium.

Results: NTPDase1 was expressed in the smooth muscle and endothelial cells, coinciding with localization of ADPase activity. NTPDase2 was largely expressed and active (ATPase activity) in ciliated cells and in connective tissue. PLAP was immunodetected and active in luminal epithelium.

Conclusions: We found that this new method is specific, sensitive, and useful with different tissues. Our results show that ecto-nucleotidases are abundantly present in human oviducts where these enzymes work in concert to metabolize extracellular ATP to adenosine. This study contributes to knowledge of purinergic signaling by ecto-nucleotidases in the female reproductive system.

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REDISTRIBUTION OF HEAT SHOCK PROTEIN A2 ON THE HUMAN SPERM MEMBRANE AFTER CAPACITATION IN VITRO

Huerta-Retamal, Natalia[1]; Sáez-Espinosa, Paula[1]; Robles-Gómez, Laura[1]; Aizpurua, Jon[2, 3]; Gómez-Torres, Mª José[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

Introduction: The heat shock protein A2 (HSPA2) correlates with sperm maturation, function and fertility. Recent work has revealed that in the infertile population, spermatozoa that fail to interact with the ZP of the oocyte consistently lack HSPA2 protein expression. The objective of this study was to know the localization of HSPA2 receptor in spermatozoa before and after capacitation and analyse the changes on its location.

Material and Methods: The samples were obtained from ten normozoospermic donors. The capacitation was carried out by swim-up for 1 hour. Assessment of the distribution of the HSPA2 receptor was performed by indirect immunofluorescence using anti-HSPA2 antibody. It was observed a minimum of 400 cells in each physiological condition by confocal microscopy and the data were analyzed using Student t distribution ($p < 0.05$).

Results: We detected 13.8% of positive spermatozoa for HSPA2 in uncapacitated sperm and 17.5% in capacitated sperm. Furthermore, we described HSPA2 receptor distribution patterns: pattern 1 (P1) without labelling; pattern 2 (P2), intense fluorescence on the lower part of the postacrosomal region; pattern 3 (P3) intense labelling in the equatorial band; pattern 4 (P4), intense fluorescence in the equatorial band accompanied by a less intense and homogeneous labelling throughout the acrosomal region; pattern 5 (P5) homogeneous fluorescence among the acrosomal region; and pattern 6 (P6), slightly fluorescence homogeneously distributed throughout the acrosomal region with a more intense labelling at the top of the acrosome. Moreover we noticed that in uncapactated spermatozoa, the significantly most abundant pattern was P2 (9.76%). However, in capacitated sperm the significantly most representative patterns were P3 (6.53%) and P5 (6.45%).

Conclusions: The low percentage of positive spermatozoa for this receptor may be because it is a marker of maturity. Our data suggest that HSPA2 receptor is modified according to in vitro capacitation. After this process, the receptor moves to the anterior region of the sperm head and it concentrates more intensely on the equatorial band, therefore we thought HSPA2 to be a receptor involved in the primary recognition of the sperm to the zona pellucida of the oocyte.

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SURVIVING DESICCATION: THE OOCYTE ENVELOPE OF ANNUAL KILLIFISHES. FROM HISTOLOGY TO GENE EXPRESSION

Nicolás Papa [1], María José Arezo [1], Cora Chalar [1], Jimena Montagne[1], Graciela Clivio[1] , Nibia Berois [1].

[1]Cell Biology Department. School of Sciences. Universidad de la República. Montevideo, Uruguay.

Introduction: Annual killifishes are freshwater teleost that inhabit shallow temporal ponds in tropical and subtropical regions of the Americas, Africa, and South-eastern Asia. The adult populations die during the dry season, while embryos survive these months. Thus, the persistence of the species is dependent on the buried embryos that hatch the next rainy season. They have a short lifespan and exhibit a set of unique developmental features. Epiboly is temporally and spatially detached from axis formation and embryos undergo reversible arrest: diapause I at the end of epiboly, diapause II at middle somite stage and diapause III at pre-hatching stage. The oocyte envelope is very strong and protects the embryos. Here we present ovary organization and deposition of the oocyte envelope (OE), reproductive strategy, ultrastructure of OE, identified ZP genes and their expression in *Austrolebias charrua*, an annual south-American killifish.

Materials and methods: Adults of *A. charrua* were collected in temporal ponds of the east of Uruguay and maintained in laboratory conditions. Gonads were processed with standard procedures for histological and ultrastructural analyses. The search for ZP genes was made by RT-PCR, their expression level was studied by qPCR and their regulation explored by exposition to estrogen and xenoestrogens.

Results, conclusions: The histological organization pattern of gonads corresponds with a female asynchronous spawning mode and a male continuous spawning. The OE is formed by deposition of layers during vitellogenesis. The fully-grown oocyte shows a trilaminar OE organization at TEM and an ornamented surface at SEM. There were identified two fragments of genes ZP: *achzpL* y *achzpH* (GenBank KP083410 and KP083411) both expressed in the liver and under hormone regulation. The obtained results go deep to know the function of OE related to the desiccation-resistance of the embryo. Furthermore, the liver expression of the genes, estrogen regulated, allows to propose the OE of annual killifishes as a reliable biomarker to monitor water contamination.

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FUNCTIONAL REPRODUCTIVE STATUS AFTER PREPUBERTAL OR ADULT OVARY GRAFT.

Rebeca Chávez[1], Gabriel Anesetti[1].

[1]Department of Histology and Embryology. Faculty of Medicine, University of the Republic, Montevideo, Uruguay.

Introduction: Ovary graft has been study as a means to restore fertility, however the existence of differences between the feasibility of graft originated in adult or prepubertal models and its repercussion on hormonal successful has been little discussed. In this case, we analyzed the effects of prepubertal or adult autograft on hormonal profile and its effects on uterine and vaginal histology at short or long time after graft.

Materials and Methods: Ovaries from prepubertal (Graft20; n=8) or adult (Graft90; n=8) rats were excised using a dorsolateral approach under ether anaesthesia. Ovaries were immediately autografted into a subcutaneous flank pouch. Animals were sacrificed 3 or 8 months later. At autopsy, ovaries uterus and vagina were obtained and morphometric, immunohistochemical and biochemical approaches were used to analyze reproductive functionality.

Results: Number of follicles, hormonal serum levels, uterus and vaginal morphometric data are in the table. No changes in percentage of estrogen receptor labeled epithelial cells were detected in any group.

Group	Follicle (n)	E2 (pg/ml)	T (ng/ml)	Uterus (µm)		Vagina (µm)	
				Endometrim thickness	Muscle thickness	Epithelium thickness	Muscle thickness
Graft 20-3m	45± 13*	80± 10	18 ± 4	626 ± 35	497 ± 26	85 ± 8	570 ± 32
Graft 20-8m	8 ± 2	47 ± 15	19 ± 3	879 ± 187	640 ± 62	172 ± 12	467 ± 36
Graft 90-3m	13 ± 6	50 ± 6	13 ± 6	557 ± 95	678 ± 35	100 ± 7	769 ± 26
Graft 90-8m	1	52 ± 7	35 ± 3*	456 ± 33	644 ± 12	157 ± 7	1234 ± 38

(*) $p<0.05$

Conclusions: Prepubertal graft promotes a bigger number of ovary follicles and estradiol secretion after shortly after ovary excision. If grafts were performed on adult period, the number of follicles reduces faster and an increase of testosterone serum levels long time after was observed.

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HISTOLOGICAL AND MORPHOCUANTITATIVE ASPECTS OF FREE CORIAL VELLOSITIES IN NORMAL PLACENTAS, WITH DIABETES AND ARTERIAL HYPERTENSION

Ruth Prieto Gómez[1], Nicolás Ernesto Ottone[2,], Cristian Sandoval[3] & Homero Bianchi [4]
1. Departamento de Pediatría y Cirugía Infantil, Facultad de Medicina, Universidad de La Frontera, Temuco, Chile.
2. CICO – Centro de Investigación en Ciencias Odontológicas, Facultad de Odontología, Universidad de La Frontera, Temuco, Chile.
3. CIMA, Facultad de Odontología, Universidad de La Frontera, Temuco, Chile.
4. Departamento de Anatomía, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.

Introduction: Gestational pathologies such as hypertension and gestational diabetes mellitus (GDM) can determine changes in the macro and microscopic morphological characteristics of the placenta and its free chorionic villi, and in the fetus it can be accompanied by pathological manifestations.

Objective: To describe morphometric and histological aspects of free chorionic villi in normal pregnancies, with diabetes and hypertension.

Material and method: Thirty human placentas were used and were separated into three groups: Normal (N), Hypertensive Pregnancy Syndrome (SHE) and Diabetes (D), according to the presence or absence of pathologies in pregnancy. Tab was used to record placental and newborn weight. All samples were fixed in 10% buffered formalin. From each, 5 samples were extracted, obtaining 25 cuts for each placenta. Subsequently, they were stained with H & E, Alcian Blue, Masson's Trichrome, PAS-Hematoxylin and PAS-Diastase. In addition, histological and morphometric analysis (ImageJ®) of the chorion villus was performed. Statistical analysis was performed using ANOVA.

Results: Among the morphological changes, an increased placental weight / weight ratio of the newborn was found in Gestational Diabetes Mellitus associated to histological changes. There were no significant morphometric changes between placentas N, SHE and D. There was an increase in the number of corial vessels in placentas of group D ($P < 0.05$) and of the surface between the chorion villi. In the SHE group there was a moderate increase in syncytial nodes and presence of fibrin in the stroma.

Conclusions: Placentas with Gestational Diabetes Mellitus experience histological alterations, as a consequence of structural and functional changes. In the case of SHE, placental alterations are related to the severity of the disease.

KEY WORDS: Placenta; Free chorionic villi; Gestational diabetes mellitus; Hypertension; Morphometry.

LECTIN BINDING-PATTERN OF GLYCOCONJUGATES DURING TESTICULAR SPONTANEOUS RECRUDESCENCE AFTER EXPOSURE TO SHORT PHOTOPERIOD IN SYRIAN HAMSTER (*MESOCRICETUS AURATUS)*

Seco-Rovira V., Martínez-Hernández, J., Beltrán-Frutos E, Ferrer C, Luis M. Pastor
Department of Cell Biology and Histology, Medical School, IMIB, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, Spain

Introduction: Changes in glycoprotein expression during spermiogenesis have been observed in diverse species of mammals and different reproduction states. Lectin histochemistry is a suitable tool for identifying germinal cells in apoptosis, at what stage of cell death and in which germinal cell types they undergo this process, especially spermatocytes and round spermatids. The objective of the present communication is to characterize the glycoconjugate pattern of hamster testis during recrudescence by means of lectin histochemical methods and to study the changes in the glycoconjugate pattern during germ cell apoptosis.

Material and Methods: Twenty-five 6-month-old male hamsters *(Mesocricetus auratus*) were used (20 subjected to a short photoperiod of 8:16 LD and 5 maintained with a 14:10 LD cycle). Eleven HRP- or digoxigenin-labeled lectins were used in testicular samples obtained during the recrudescence process. Three recrudescence groups were established (Initial Recrudescence (IR), Advanced Recrudescence (AR) and Total Recrudescence (TR)).

Results: During recrudescence, the seminiferous epithelium and Leydig cells gradually recovered the glycoconjugate pattern to reach similar values to those found in the testes not subject to short photoperiod. This was particularly evident from the affinity of lectins (PNA, GNA, AAA, Con-A and LTA) for germ cells in apoptosis. These lectins showed decreased affinity as testicular recrudescence advanced, indicating a possible decrease of germ cells in apoptosis.

Conclusions: The alterations observed in the pattern of glycoconjugates in the regressed testis due to the exposure to a short photoperiod disappeared quickly and gradually during recrudescence. In addition, the recovery of the seminiferous epithelium with the reestablishment of spermatogenesis is accompanied by a decrease in the loss of cells of the seminiferous epithelium by apoptosis.

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ECTO-NUCLEOTIDASE EXPRESSION AND ACTIVITY IN ENDOMETRIOID-TYPE ENDOMETRIAL CARCINOMA CELL LINES

Aitor Rodríguez-Martínez[1,2], Xavier Matias-Guiu[2,3], Mireia Martín-Satué[1,2]

[1]Departament de Patologia i Terapèutica Experimental, Facultat de Medicina i Ciències de la Salut, Campus Bellvitge, Universitat de Barcelona, Spain
[2]Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), Spain
[3]Servei d'Anatomia Patològica, Hospital de Bellvitge, Barcelona, Spain

Introduction: ATP and adenosine are known for their role in promoting an immunosuppressive environment at the tumor site. It is known that some ecto-nucleotidases, proteins that handle the hydrolysis of tri-, di- and monophosphate nucleotides, are overexpressed in endometrial tumor tissues although the mechanisms underlying these processes remain controversial. In the present study we characterized, with cytochemistry and i mmunofluorescence, the ecto-nucleotidase profiles in endometrioid-type endometrial carcinoma cell lines. Furthermore, we have developed a new approach capable of simultaneously detecting both alkaline phosphatase expression and activity.

Materials and methods: *Cell lines*: 3 endometrioid endometrial carcinoma cell lines (Ishikawa, HEC-1B and ECC-1) were used for cytochemistry and immunofluorescence experiments. *In situ ecto-nucleotidase enzyme activities:* ATPase, ADPase, and AMPase activity experiments were performed in all cell lines, based on the Wachstein/Meisel technique. *Immunofluorescence experiments:* cell immunolabeling was performed using primary antibodies against different members of the ecto-nucleotidases: anti-ectonucleoside triphosphate diphosphohydrolase 2 (E-NTPDase2), anti-E-NTPDase3, anti-CD73, and anti-placental-like alkaline phosphatase (PLAP). *Alkaline phosphatase immunoactivity assay:* for the detection of both activity and al kaline phosphatase expression we developed a combinatorial assay in which enzyme activity can be co-visualized with protein expression. Immunofluorescence followed by an activity assay was performed on AP-expressing cells.

Results: Cytochemistry enzyme assays showed moderate ATPase activity in both Ishikawa and ECC-1 cell lines. Moderate ADPase activity was found in ECC-1 cells and high AMPase activity was detected in HEC-1B cells. In immunolabeling experiments we found NTPDase2 protein expression in all three cell lines, with NTPDase3 expression restricted to ECC-1 cells. CD73 was detected in all three cell lines. Moderate alkaline phosphatase activity and protein expression were found in Ishikawa and ECC-1 cells.
A wide differential range of activities and ecto-nucleotidase expression among the studied cell lines was observed. NTPDase2 and NTPDase3 expression correlated with ATPase activity. CD73 protein expression coincided with the high AMPase activity shown with HEC-1B.

Conclusions: Ishikawa, HEC-1B, and ECC-1 cell lines represent a useful cell model for the study of ecto-nucleotidases in the context of endometrioid-type endometrial carcinoma.

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ANALYSIS OF THE IMPORTANCE FACTOR IN THE FISH *SCOMBEROMORUS CAVALLA* OF TECOLUTLA, VERACRUZ, MEXICO

Alejandra Reyes Márquez[1], Marcela Arteaga Silva[2], Gerardo Figueroa Lucero[3], Irma Hernández Calderas[1], Felipe de J. Muñoz-González[1], Xochitl Guzmán García[1].

[1]Departamento de Hidrobiología. División de Ciencias Biológicas y de la Salud. Laboratorio de Ecotoxicología, Histopatología. Universidad Autónoma Metropolitana Unidad Iztapalapa. Ciudad de México, México.
[2]Departamento de Biología de la Reproducción. División de Ciencias Biológicas y de la Salud. Laboratorio de Neuroendrocrinología. Universidad Autónoma Metropolitana Unidad Iztapalapa. Ciudad de México, México.
[3]Departamento de Hidrobiología. División de Ciencias Biológicas y de la Salud. Planta Experimental de Producción Acuícola. Universidad Autónoma Metropolitana Unidad Iztapalapa. Ciudad de México, México.

Introduction: In the environmental monitoring it has been pointed out the importance of using fish as indicator of the quality of the medium, due to it's breadth of biological responses and sensitivity to stressors. Exposure to contaminants is frequently associated with a series of lesions or alterations in different organs that can be evaluated through histopathological analysis. It has been widely proposed in the analysis of tissue responses in fish the use of health indexes that relate the factor of pathological importance and the intensity of the observed responses. The aim of this was to evaluate histopathological responses in two organs (liver and muscle) of *S. cavalla*, associating a pathological Importance factor.

Materials and methods: The study was conducted in Tecolutla, Veracruz, Mexico. Analyzed organs were embedded in paraffin, cut (5 µm) and stained with H-E. A total of 90 slates (N = 10) were observed with an optical microscope. A prevalence of histophatological alterations was constructed to recognize the frequency of occurrence per alteration and were categorized in order from lowest to highest pathological impact. From the categorization of tissue responses, an Importance Factor (FI), (0-3) and reaction intensity (0-6) were assigned according to Bernet *et al*. (1999). The data were treated statistically to recognize the degree of tissue involvement.

Results: The main tissue responses observed in the liver were: congestive vessels, focal infiltrations, inflammations, eosinophilic secretions, melano-macrophage centers and granulomas; which are described as lesions with IF between 1 and 3 (minimal and severe pathological importance). Tissue responses observed are associated with protection mechanisms (inflammation, eosinophilic secretions), circulatory disturbances (congestive vessels and infiltrations) and with immunological mechanisms (melano-macrophage centers). In relation to the muscle, only circulatory disturbances (congestive vessels) were observed.

Conclusions: The standardization of the alterations asigned from the pathological reference categories allows a quantitative evaluation of the health status of organisms. Lessions observed in *S. cavalla* where of the reversible type, therefore it is recommended to continue with biomonitoring studies that evaluate the presence of contaminants in Tecolutla, Veracruz.

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MORPHOMETRIC ALTERATIONS IN ANIMAL MODELS OF CHRONIC HYPERAMMONEMIA

Ruiz-Saurí A.(1,4), Frances, E. (1), Balzano T (2), Perez de la Cruz, MA. (3), Felipo V. (3), Montoliu C (1,4)

(1) Pathology Department, Faculty of Medicine and Odontology, University of Valencia, Spain

(2) Laboratory of Neurobiology, Príncipe Felipe Research Center, Valencia, Spain

(3) Anatomy and Histology Department, Faculty of Medicine. University of Salamanca, Spain

(4) INCLIVA-Health Research Institute, Valencia, Spain

Introduction: Hepatic encephalopathy (HE) is a neuropsychiatric syndrome present in patients with liver disease. There are animal models that allow reproducing this pathology: hepatic encephalopathy model by porta-cava anastomosis (PCS), by Biliary Ligature (BDL) and model of chronic hyperammonemia without hepatic failure (HA). We have been studied the repercussion of hepatic encephalopathy in hippocampal and cerebral cortex neurons.

Material and Methods: We studied 55 brains from Wistar Albinas rats, divided into three experimental groups according to the HE model used, together with their respective sham controls. The sacrifices were made at different times after surgery or the beginning of the diet. it has been at 3 days and 2, 4 and 8 weeks. The samples were fixed in formalin, stained with Hematoxilin and Eosin and scanned. Then, we selected with Panoramic Viewer 3 zones of the hippocampus and 3 of the cortex at 19.36 magnifications. We performed a morphometric analysis with Image-ProPlus 7.0 to analyze different parameters of the neurons: area, major axis, minor axis, maximum diameter, fractal dimension, heterogeneity, IOD, perimeter and roundness. Student t-test or Anova for continuous variables and Chi-Square (χ^2) for categorical variables were performed.

Results: First we find that the area of the neurons decreases in the cortex and the perimeter and fractal dimension decrease in the hippocampus with the hyperammonemia, in the three study groups. This difference is statistically significant. Second, we found that the neurons of the cortex are larger than those of the hippocampus in both controls and in rats with hepatic encephalopathy. Finally, comparing the results according to the evolution over time, we have found that the neurons are decreasing in size (decrease of roundness and fractal dimension in hippocampus, decrease of IOD and increase of heterogeneity in cortex) as the ammonium exposure increases in the HA group.

Conclusions: From the morphometric point of view, we have shown that in hepatic encephalopathy, there are not only changes in glia cells but also there are changes in the size and shape of neurons as the time of exposure to ammonium increases, in a murine experimental model of chronic hyperammonemia.

WOUND HEALING PROPERTIES OF TOPICAL POLYACETAL AND HYALURONIC ACID - A HISTOLOGICAL STUDY IN A MURINE MODEL

Ruiz-Saurí, A. (1,2), Garcia, N. A. (3,4), Rodríguez-Escalona, G. (5), Monleón Pradas, M. (6), Vicent, M.J. (5), Sepúlveda Sanchís, P. (3, 4), Marcos-Garcés, V. (1),

(1) Pathology Department, Faculty of Medicine and Odontology, University of Valencia
(2) INCLIVA Biomedical Research Institute, Valencia
(3) Medical Research Institute Hospital La Fe (IIS La Fe), Valencia
(4) Cardiovascular Repair Laboratory, Príncipe Felipe Research Center, Valencia
(5) Polymer Therapeutics Laboratory, Príncipe Felipe Research Center, Valencia
(6) Center for Biomaterials and Tissue Engineering, Polytechnic University Valencia

Introduction: Enhancement of skin wound healing with biopolymers is a new field of research that has provided promising results. Polyacetal (PA) has many applications in tissue engineering, but its usefulness in skin regeneration hasn't been studied yet. In a murine model, we studied the regenerative properties of topical hyaluronic acid (HA) with or without PA.

Material and Methods: We performed a 5mm diameter punch biopsy in C57BL6 mice aged 8-10 weeks. Afterwards, a topical treatment was applied - saline solution (control group, n=16) or a 50 µl water resuspension of HA with or without PA (each n=16). Animals were sacrificed at day 3, 10 and 22 and samples were stained with Hematoxilin-Eosin and Masson's trichrome. We took 5 microphotographs with a Leica DMD108 microscope and measured different parameters with the IMAGE-PRO PLUS 7.0 software. Student t-test for continuous variables and Chi-Square (χ^2) for categorical variables were performed.

Results: All groups had an intense inflammatory infiltrate, full-thickness skin defect and scab formation at day 3. A complete regeneration was achieved by day 22, without differences between groups.

Topical HA increased scar width (day 3) and reduced epidermal hypertrophy (day 10). However, HA-PA reduced scar width (days 3 and 10) and recovered normal interdigitation index and epidermal thickness (day 10). A milder inflammatory infiltrate was seen in PA-HA and HA-alone groups (day 10), consistent with lower cellular density, and regeneration of panniculus carnosus muscle was evident only in these groups (day 22).

A thicker reticular dermis and thicker collagen bundles were seen in HA-alone group (day 10). In HA-PA group, less collagen density and more parallel collagen bundle orientation were seen in the reticular dermis (day 10).

Conclusions: Histopathological data show that a single application of topical PA-HA has beneficial effects on murine skin healing after full-thickness skin defects, as demonstrated by an accelerated wound closing, less inflammatory infiltrate, faster recovery of normal epidermal thickness and dermo-epidermal junction interdigitation and a probable modulation of collagen synthesis in the reticular dermis. Most of these changes were not present in HA-alone group, suggesting that PA addition has a beneficial effect on skin wound healing.

HISTOLOGY, MORPHOMETRIC AND IMMUNOHISTOCHEMISTRY STUDY OF CONDUCTION SYSTEM OF THE HEART IN PIGS.

A. Ruiz-Sauri (1,2), Garcia-Bustos V. (1), Izquierdo M. (2,3), Molina P. (1), FJ. Chorro FJ. (2,3), Rios C (3), Bodí V. (2,3), Sebastian R. (4)

(1) Department of Pathology, School of Medicine, Universitat de Valencia, Valencia, Spain
(2) INCLIVA Biomedical Research Institute, Valencia, Spain
(3) Cardiology Department Hospital Clinico Universitario de Valencia, Valencia, Spain
(4) Computational Multiscale Simulation Lab, Universitat de Valencia, Valencia, Spain

Introduction: The anatomy of the cardiac conduction system (CCS) has been widely studied. However, subsequent efforts have mainly addressed suprahisian structures owing to its clearer implications in supraventricular rhythm disorders and the complexity of the CCS at distal sections. In this work, we study the distribution and morphological properties of the Purkinje network with special emphasis in the cellular and architectural characterization of its intramural branching structure, mesh-like subendocardial network, and the Purkinje myocardial junctions in adult pig hearts by means of both histopathological and morphometric evaluation.

Material and Methods: The hearts of two young pigs and two adult human autopsies with no signs of cardiac pathology have been used. The material has been fixed with 4% formaldehyde buffered and processed with conventional histological techniques. All sections have been stained with H.E. And trichrome and immunostaining with anti-Connexin 40, anti-Desmin and anti-CD56. Digital images have been obtained with the Leica DMD108 microscope and these have been subsequently analyzed morphometrically with Image-Pro Plus RESULTS

Results: In general, the histological and morphometric characteristics of the conduction system of both species are similar (the purkinje fiber bundle thickness decreases according to the base order, middle third, apex). In order to assess the proper electrical correlate, the PMJs were estimated combining the histological features under hematoxylin-eosin as well as the pattern of expression of Cx40 by immunohistochemistry. Regarding the regional distribution, PMJs were present in 47.1% of the micrographs of the posterior region, 53.8% in the septal region, 56.9% in the anterior region and 58.8% in the lateral region; and the densities were 0.33, 0.41, 0.43 and 0.46 PMJs per square millimetre respectively.

Conclusions: We have stablished the first morphometric study of the Purkinje system and provided objective data which should be considered in the development of these models, beyond gross and variable pathological descriptions. Our findings on PC distribution, regional characteristics and histological and immunohistochemical study of PMJs strongly provide an anatomical explanation for the electrical impulse spread through the Purkinje network, and, over further studies, could be useful in the characterisation of pathological processes.

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DOES IT HAVE PROGNOSTIC VALUE THE STUDY OF THE VASCULAR PATTERN IN RENAL CELL CARCINOMAS? ANALYSIS THROUGH A MORPHOMETRIC STUDY.

Ruiz-Saurí, A. (1,2), García-Bustos, V. (1), Granero E. (1), Cuesta S.(1), Sales (MA.(3), Marcos V.(1), MA Perez de la Cruz (MA.(4), Rius C (5), Bodí V. (2,5), Llombart-Bosch A.(1)

(1).Pathology Department. School of Medicine. University of Valencia. INCLIVA. Valencia, Spain.
(2). INCLIVA. Biomedical Research Institute. Valencia, Spain
(3). Service of Pathology. University Clinical Hospital, Valencia, Spain
(4). Department of Histology. School of Medicine. University of Salamanca, Spain
(5) Cardiology Department Hospital Clinico Universitario de Valencia, Valencia, Spain

Introduction: The significance of microvascular density in renal carcinoma are limited, despite the large number of papers that have been published is controversial. Moreover, little evidence has been published about the development of tumor vascularization from the objective point of view of morphometry. The aim of this study was propose a classification of renal cancer tumor blood vessels according to their morphology and vascular pattern see by morphometric study.

Material and Methods: Tissue samples were obtained from 121 renal cell carcinoma. 101 out of 121 cases were Renal Cell Clear Cell Carcinoma (CCRCC), 10 cases were Papillary Renal Cell Carcinoma (PRCC) and 10 cases were Chromophobe Renal Cell Carcinoma (ChrRCC). In addition, we studied five samples of healthy renal tissue which served as a control group. By study histology and morphometric vascular pattern we staining with H-E and anti-CD31 We quantified the microvascular density, microvascular area and different morphometric parameters: maximum diameter, minimum diameter, major axis, minor axis, perimeter, radius ratio and roundness

Results: We identified four vascular patterns: pseudoacinar, fascicular, reticular and diffuse. Pseudoacinar and fascicular patterns were more frequent in clear cell renal cell carcinoma (37.62 and 35.64% respectively), followed by reticular pattern (21.78%), while the diffuse pattern was mainly seen in chromophobe tumors (60%). The isolated pattern was present in all papillary tumors (100%). In healthy renal tissue, the pseudoacinar and isolated patterns were differentially found in the renal cortex and medulla respectively.

Conclusions: We defined four distinct vascular patterns significantly related with the ISUP tumor grade in renal cell carcinomas. Further studies in larger series are needed in order to validate these results.

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IMPLICATIONS AND MECHANISTIC ROLE OF EOSINOPHILS AFTER REPERFUSED MYOCARDIAL INFARCTION. STUDY IN PATIENTS AND IN VIVO

Amparo Ruiz-Sauri[1], Cesar Rios-Navarro[2], Jose Gavara[2], Arantxa Hervas[2], Nerea Perez-Sole[2], Elena de Dios[2], Nuria Daghbouche-Rubio[2], Francisco J. Chorro[2], Vicente Bodi[2]

[1]Pathology Department. School of Medicine. University of Valencia. INCLIVA. Valencia, Spain.
[2]Cardiology Department. Hospital Clinico Universitario de Valencia. University of Valencia. INCLIVA. Valencia, Spain.

Introduction: Deregulation of immune system has been traditionally associated with extensive infarct size and adverse cardiac events after myocardial infarction (MI). However, little attention has been paid to eosinophils, a potent subset of pro-inflammatory innate immune cells. We aimed to elucidate the implication of eosinophils after MI in three different scenarios: 1) the time-course in serum of patients with MI. Histological determination of eosinophils in 2) myocardial samples from a swine model of acute MI and 3) in myocardial autopsies from patients with chronic MI.

Material and Methods: 1) A prospective study that involved 520 patients with a first ST-segment elevation MI was performed. Serial measurements of circulating eosinophil counts were analysed at admission and at 12, 24, 48, 72, and 96h. 2) Swine model of MI was performed by transitory 90-min occlusion followed by 3-days, 7-days and 1-month reperfusion. The presence of eosinophils in the infarcted myocardium was histologically quantified by immunohistochemistry using anti-major eosinophil cationic protein and by Luna´s staining, specific for eosinophil granules. 3) In human samples of autopsies from chronic MI patients, eosinophils were also assessed.

Results: 1) Eosinophil count dramatically decreased 12h after reperfusion in comparison to the arrival; afterwards, it progressively increased until reaching its maximum value 96h after reperfusion. 2) In porcine myocardial samples, the number of eosinophils peaked 3-days (312.78 cells/mm^2) and 7-days (254.09 cells/mm^2) after reperfusion, while being reduced 1-month after reperfusion (127.04 cells/mm^2 vs control: 60 cells/mm^2). 3) In samples obtained from autopsies, the presence of eosinophils was also detected in the myocardium from chronic MI patients (305.51 cells/mm^2 vs. control: 35 cells/mm^2).

Conclusions: Eosinophils might be involved in the pathophysiology of reperfused MI. The decrease in circulating eosinophil count after reperfusion mirrors their migration into the infarcted myocardium, as reflected by the presence of eosinophils in heart samples from swine and patients. Further studies are needed to understand the pathogenic role of eosinophils in this context.

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INHOMOGENEOUS ORGANIZATION OF COLLAGEN WITHIN THE FIBROTIC SCAR AFTER MYOCARDIAL INFARCTION. RESULTS IN A SWINE MODEL AND IN PATIENTS

Amparo Ruiz-Sauri[1], Arantxa Hervas[2], Cesar Rios-Navarro[2], Elena de Dios[2], Jose Gavara[2], Nerea Perez-Sole[2], Francisco J. Chorro[2], Vicente Bodi[2]

[1]Pathology Department. School of Medicine. University of Valencia. INCLIVA. Valencia, Spain.
[2]Cardiology Department. Hospital Clinico Universitario de Valencia. University of Valencia. INCLIVA. Valencia, Spain.

Introduction: The infarct healing process is implicated on left ventricular remodelling and the occurrence of cardiac arrhythmias after myocardial infarction (MI). Collagen organization within the fibrotic scar following MI has not been clearly defined. We aimed to characterize the organization of collagen fibers within the fibrotic scar in a swine model and in human samples from patients with chronic MI.

Material and Methods: MI was induced in swine by transitory 90-min occlusion by using angioplastic balloons followed by 1-week (acute MI group) or 1-month (chronic MI group) reperfusion. Infarcted myocardial samples obtained from swine and from patients with chronic MI were obtained and endocardium, epicardium, peripheral area and core area were studied. After staining the samples with picrosirius red method, the organization of the collagen fibers was quantified by using Fast Fourier Transform (higher values of the collagen organization index indicate higher disorganization) was studied in 100 swine samples and 95 human samples. Moreover, collagen distribution was corroborated by transmission electron microscopy (TEM) and by immunohistochemistry using anti-a-smooth muscle actin for myofibroblast detection.

Results: In the experimental model, no differences in collagen organization were found between the acute and chronic groups in the core area of the scar. In the chronic group, the endocardium [0.90 (0.84–0.94)], epicardium [0.84 (0.79–0.91)] and peripheral area [0.73 (0.63–0.83)] displayed a disorganized pattern in comparison with the core area [0.56 (0.45–0.64)]. Indeed, those results were confirmed in TEM captions and immunohistochemistry analysis from animal samples. Similarly, in human autopsies from patients with chronic MI, collagen fibers were more disorganized in all outer areas than in the core area ($p<0.0001$).

Conclusions: In a controlled experimental model and in patient samples, collagen fibers are organized almost in a parallel manner in the core area whereas in the endocardium, epicardium and peripheral areas collagen fibers show a random arrangement. This finding contributes pathophysiological information regarding the healing process; furthermore, it may lead to elucidate the genesis and invasive treatment of arrhythmias post-MI.

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C57BL/6 MICE MULTIPLE SCLEROSIS CUPRIZONE MODEL: ADITIONAL SYSTEMIC FINDINGS

Ana Navarro-Incio[1 2], María Álvarez-García[1], Gemma Fernández-García[1], Sandra Villar-Conde[1], Enrique García-Álvraez[1], Eva del Valle-Suárez[12], Eva Martinez-Pinilla[12], Jorge Tolivia-Fernández[12]

[1]Department de Morphology and Cell Biology, Faculty of Medicine and Health Sciences, University of Oviedo, Spain

[2] Instituto de Neurociencias de Principado de Asturias (INEUROPA), Spain

Introduction: Cuprizone mice model is commonly used in multiple sclerosis to study white matter experimental de- and remyelination of the corpus callosum and cerebellar peduncles. One the preferred strain for the application of this model is the C57Bl/6 in which is extendedly proved that the administration of cuprizone causes cell death of oligodendrocytes and subsequently demyelination. Another feature in this animal model is that most mice develop edema and hydrocephalus. Weight reduction and certain mortality rate were noted during feeding period. However, very few data on cuprizone systemic effect have been reported. The aim of this work was to study the toxicity of this cupper chelator in organs that could be implicated in detoxification and hydric equilibrium.

Material and Methods: Eight weeks old C57BL/6 mice were caged individually and fed with standard grounded chow containing 0.2% w/w cuprizone for 3 and 6 weeks. The mice were weighted every two days and the water consumption was measured. Animals were then sacrificed and necropsies were carried out. Some organs such as brain, liver and kidney were later processed for observation under photonic and electronic microscopy.

Results: Weight lost was only observed in the mice fed with cuprizone for three weeks. Water consumption rate was affected in all experimental groups when compared with controls. Cuprizone mice showed a loss of muscle mass and fatty tissue, and the bladder and gallbladder appeared swollen. Other organs did not present qualitative changes, except for the weight of brain, liver and kidney that were greater in mice exposed to cuprizone for 3 weeks than in controls. Photonic microscopy analysis did not show fibrosis, inflammation or necrosis in any studied organs. However, electron microscopy showed slight mitochondrial changes in liver and kidney and an increase of residual bodies.

Conclusion: These data demonstrate that apart from brain, other body organs exhibit changes due to cuprizone toxicity. These changes could be related to a slight edema produced by deregulation of the hydric balance. Furthermore, we have found evidences to mitochondrial damage in liver and kidney as it has been already reported for oligodendrocytes.

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MITOPHAGY ACTIVATION IN BLOOD MONONUCLEAR CELLS AS A POSSIBLE BIOMARKER FOR THE DIAGNOSIS OF FIBROMYALGIA

AM. Moreno-Fernández[1], L. Macías-García[1], D. Cotán[2], JA. Sánchez-Alcázar[2], A. Fernández-Rodríguez[1], M. de Miguel-Rodríguez[1].

[1]Department of Cytology and Histology Normal and Pathological. Faculty of Medicine. University of Seville, Seville, Spain.
[2]Andalusian Center on Developmental Biology (CABD-CSIC),University Pablo de Olavide and Networking Biomedical Research Center on Rare Diseases (CIBERER), Seville, Spain.

Introduction: Fibromyalgia (FM) is a common chronic pain syndrome. The newer 2010 American College of Rheumatology (ACR) diagnostic criteria define FM as a chronic widespread pain condition associated with fatigue, sleep and cognitive disturbance and a variety of somatic symptoms (1). Oxidative stress markers have been proposed as a relevant event in the pathogenesis of this disorder (2, 3). Moreover, we have detected mitochondrial dysfunction and it was also associated with increased expression of autophagic genes and the elimination of dysfunctional mitochondria by mitophagy (4, 5). Mithocondrial dysfunction and mitpohagy have also been detected in other disorders with muscular and neurological clinical presentations, such as Parkinson (6), congenital muscular distrophy (7) and MELAS syndrome (8).

Material and Methods: We studied blood mononuclear cells (BMCs) of 34 patients with FM. Our patients, 32 women between 30-80 years old and 2 men, 37 and 47 years old, were diagnosed with FM by exclusion of other diseases and syndromes and in accordance with the ACR criteria. All of these patients had daily episodes of intense musculoskeletal pain and fatigue, stiffness, anxiety, sleep disturbance and depression. They all presented high scores in the Visual Analogical Scale of pain (VAS) and in the Fibromyalgia Impact Questionnaire (FIQ). BMCs from heparinized blood were purified with isopycnic centrifugation by using Histopaque-1119 and Histopaque-1077 (Sigma Chemical Co.). Autophagy markers (MAP1LC3-beta,Microtubule-Associated Proteins 1, Light Chain 3β) were isolated using Western Blot.

Results: Results in this study demonstrated an association between the presence of mithocondrial dysfunction and mitophagy in BMCs in 86,7% of the 34 patients we studied. These results were confirmed by electron microscopy that clearly showed autophagosomes where mitochondria were being degraded. 13 patients of the total patients studied showed mitophagy greater than 200%. The greater mitophagy percentage was 893%. Only 3 patients studied didn´t show mitophagy (value lower than 100%).

Conclusions: We present mithocondrial dysfunction associated to mitophagy as a possible biomarker for FM diagnosis.

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PD-L1 IS A PREDICTIVE FACTOR FOR RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN TRIPLE NEGATIVE BREAST CANCER

Aroa Mayán[1], Ángel Vázquez-Boquete[2], Beatriz Fernández-Rodríguez[2], Teresa Curiel[3], María Paz Santiago[4], Dora Ínsua[2], María Otero[5], Francisco Gude[5], Máximo Fraga[2], José Antúnez[2], Tomás García-Caballero[1,2]

[1]Department of Morphological Sciences, School of Medicine and Dentistry, University of Santiago, Santiago de Compostela, Spain.
[2]Department of Pathology and [3]Department of Oncology, University Clinical Hospital, Santiago de Compostela, Spain.
[4]Department of Pathology, University Hospital Complex of A Coruña, Spain.
[5]Health Research Institute of Santiago, Santiago de Compostela, Spain.

Introduction. Triple negative carcinoma (TN) represents the breast cancer subtype with poor prognosis and its treatment is reduced to citotoxics due to the absence of personalized therapies. PD-L1 is a ligand of PD-1 (Programmed cell Death 1). It is present in tumor and inflammatory cells and acts blocking the immune system. The aims of the present work were: 1) To analize by immunohistochemistry the expression of PD-L1 in TN breast cancer; 2) To compare the results obtained by use of 22C3 and 28-8 antibodies; and 3) To correlate PD-L1 expression with anatomoclinical factors, pathological response grade after neoadjuvance and specific survival.

Material and methods. Fifty TN breast cancer cases treated with neoadjuvant chemotherapy were selected from the files of Pathology Departments of University Clinical Hospitals of Santiago de Compostela and A Coruña. PD-L1 expression was analyzed by immunohistochemistry using 22C3 and 28-8 pharmDx monoclonal antibodies (Dako-Agilent, Carpinteria, CA). In order to achieve a larger series for the study of prevalence of PD-L1 in TN breast carcinomas, 50 new cases without neoadjuvancy were selected and analyzed by 22C3 antibody.

Results. PD-L1 showed plasma membrane immunoreactivity both in tumor and accompanying inflammatory cells. Positivity in tumor cells was found in 21% of cases and in inflamatory cells, in 83%. The results obtained using 22C3 and 28-8 monoclonal antibodies showed an excellent concordance (94%, k=0.848; $p<0.001$). We observed correlation between the percent of immunostained tumor cells and the intensity of immunoreactivity ($p=0.001$), as well as between both parameters and the positivity in inflammatory cells ($p=0.019$). PD-L1 expression in tumor cells correlated with pathological response after neoadjuvancy with both antibodies studied (22C3: Rho=0.331, $p=0.019$, and 28-8: Rho=0.292, $p=0.039$), whereas positivity in inflammatory cells correlated whith poor prognosis pathological factors and with specific survival for 28-8 ($p=0.016$).

Conclusion. Expression of PD-L1 in tumor cells was found in 21% of TN cases and in inflammatory cells in 83%. An excellent concordance was found between both antibodies employed. Although studies in greater series are warranted, we can conclude that PD-L1 represents a predictive factor for response to neoadjuvant chemotherapy treatment in TN breast cancer.

HISTOLOGICAL, HISTOCHEMICAL, IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL CHARACTERISTICS OF NORMAL AND ABNORMAL REGENERATING SKELETAL MUSCLE FIBERS.

Fernando Leiva-Cepas[1,2,3], Ignacio Ruz-Caracuel[1,2], María Ángeles Peña-Toledo[2], Miguel Ángel Gómez-Luque[1], Ignacio Jimena[1,2,3], Evelio Luque[1,3], Rafael Villalba Montoro[4], José Peña [1,2,3]*

[1]Department Morphological Sciences, Section of Histology, Faculty of Medicine and Nursing, University of Córdoba, Spain.
[2]Research Group in Muscle Regeneration, University of Córdoba, Spain.
[3]Maimónides Institute for Biomedical Research IMIBIC, Reina Sofia University Hospital, University of Cordoba. Spain.
[4]Regional Blood Transfusion Center and Tissue Bank Sector. Córdoba. Spain.

** Present address: Department of Pathology. La Paz University Hospital, IDIPAZ. Madrid.Spain.*

Introduction: After skeletal muscle injury, regenerative muscle fibers have microscopic features indicative of maturation and growth during regeneration. In our opinion, some changes observed in human pathologic muscle can be explained by the alteration of normal muscle fiber regeneration. The aim of this study was to determine the possible modifications that the regenerative muscle fiber can undergo in an altered microenvironment causing an abnormal regeneration.

Material and methods: Normal muscle regeneration was induced in Wistar rat by intramuscular injection of a local anesthetic. Abnormal regeneration was induced by a volumetric loss of skeletal muscle. In both cases rats were sacrificed at 7 days after injury. Muscles were examined using light (cryosections were stained using histological, histochemical and immunohistochemical techniques) and transmission electron microscopy.

Results: There are clear differences in the microscopic features between the regenerative muscle fibers of the two groups. Muscle fibers with abnormal regeneration showed several cytoarchitectural changes that included fibers with myonuclei, clumps, ringer fibers, snake-coiled, central spot, split and fragmented muscle fibers.

Conclusions: Because a number of our findings are present in dystrophic and other myopathies, we think our findings may be relevant to explain the pathogenesis of some of these changes.

KIDNEY AND SKIN EFFECTS OF METALS CONTAINED IN TATTOO INKS IN RABBITS *Oryctolagus cuniculus* (Linneo 1758).

Garrido Fariña German Isauro [1] y Díaz Menchaca Karla de Jesús[1].

1. Laboratorio de Apoyo a Histología y Biología, Departamento de Ciencias Biológicas, Facultad de Estudios Superiores Cuautitlán (FES-C), Universidad Nacional Autónoma de México, Km. 2.5 Carretera Cuautitlán-Teoloyucan, San Sebastián Xhala Cuautitlán Izcalli, Edo de Méx CP 54740. México. e-mail: isaurogafa@yahoo.com.mx

INTRODUCTION: The art of tattooing began at the bronze age, it is a worldwide and increasing practice among adolescents. In different animals is used as an identification system. The ink particles are deposited by injection, endocyted and remain in keratinocytes, fibroblasts and macrophages, these particles can be found along the dermo-epidermal border, under a layer of granulation tissue. Studies in rabbits argue that fibroblasts are responsible for stabilizing the tattoo intracutaneously. Most inks have metals, either as chromogen or as a contaminant, mainly lead. The levels of acute intoxication and adverse effect on the kidney or skin are not known, renal disease may be asymptomatic until in later stages have signs and symptoms. This work explores the acute consequences of the application of tattoos, in rabbit kidney and skin for slaughter and the best choice for tattooing rabbit breeders.

MATERIALS AND METHODS: 7, 30-day-old rabbits were sedated with Xylazine/Zoletil. The inguinal area was shaved, disinfected and tattooed with only one color ink: red, green, yellow, blue, purple, black ink and chinese ink. No lesion was observed. The animals were sacrificed in the Faculty's meat shop at 120 dpi, kidney samples and tattooed skin were processed by the routine paraffin inclusion method, cut at 4 micrometers thick and stained with hematoxylin eosin, were observed in light field, phase contrast and dark field.

RESULTS: The amount of ink applied was not sufficient to achieve any observable renal pathology. The ink particles, contrary to what is thought, diffuse through the skin in an extensive way.

CONCLUSIONS. The wide diffusion of the ink into the subcutaneous tissue could explain the aggressive and morbid injuries after a tattoo session, mainly when contaminated or poor quality material are use, or the patient are susceptible to some component of the ink. The identification of rabbits is best when using trademarks ink of good quality, dark tones induce less diffusion.

DIFFERENTIAL EXPRESSION OF DISCOIDIN DOMAIN RECEPTORS IN THE TUMOR STROMA OF DUCTAL INVASIVE CARCINOMA PATIENTS.

Romayor I[1], Barcelo JR[2], Benedicto A[1], Arteta B [1].

[1]Dpto. Cell Biology and Histology, School of medicine and Enfermery, University of Basque Country (UPV/EHU), [2]Medical Oncology Service, Basurto University Hospital (HUB).

Introduction: Breast cancer is the most common carcinoma in women worldwide, being the ductal invasive carcinoma (DIC) the one with the highest incidence. Due to the implication of tumor microenvironment in cancer development, the study of stromal features could further characterize the DIC, as prognostic and therapeutic tools. Among receptors expressed in the stromal compartment Discoidin Domain Receptors (DDR-1 and -2), have been linked with numerous human cancers. However, the differential expression of DDRs in the tumor microenvironment from DIC tissues and its association with clinic and histopathological data remain poorly defined. Thus, the aim of the present study is to characterize DDRs expression in microenvironment compartments of samples from DIC patients according.

Material and Methods: To do so, samples collected from patients suffering of DIC and adjacent normal breast tissue were obtained from a total of 25 patients for histological analysis (hematoxylin/eosin staining), analysis of the expression of DDR1/2, α-SMA (fibroblasts) and CD-68 (macrophages) by immunohistochemistry, and matrix deposition analysis by Picric-sirius red staining was performed by immunochemistry. These biomarkers were related to clinical features, including tumor size, grade and stage, lymph nodes invasion and e-cadherin and HER-2 levels.

Results: A differential expression of DDR1 and DDR2 was detected not only between healthy and tumor tissue, also this expression showed different patterns of expression in tumor cells and the tumor stroma. Comparing to healthy tissues, carcinoma cells show a variable DDR1/2 expression, while increase significantly in the tumor stroma. Also, the deposition of collagen was higher in tumor stroma in associated with areas of ASMA positive cells, and the amount of non collagenous extracellular matrix was associated with areas of lower DDRs expression in tumor cells. Also, CD68 expression was increase in the tumor stroma comparing with the healthy one.

Conclusions: These results show a differential expression of DDR1/2 in the tumor and stromal area correlated with a type of tumor cells and extracellular matrix deposition. Further studies will confirm if DDR1/2 may account as a potential marker of disease subtype, progression, and development of “de novo” therapy resistance and a therapeutic target for patients suffering from DIC.

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EPENDIMOCYTES AND MORPHOSTRUCTURAL LINKAGE WITH VENTRICULAR DILATION IN SCHIZOPHRENIA

Jorge Eduardo Duque Parra [1,2]. Genaro Morales Parra [2]. John Barco Rios [1]. Jhonny Fernando García Aguirre [1,2,3].

[1] Departamento de Ciencias Básicas, Facultad de Ciencias para la Salud, Programa de Medicina, Universidad de Caldas, Colombia.
[2] Departamento de Ciencias Básicas, Facultad de Ciencias para la Salud, Programa de Medicina, Universidad de Manizales, Colombia.
[3] Departamento de Ciencias Básicas biológicas, Facultad de Ciencias para la Salud, Universidad de Autónoma de Manizales, Colombia.

Introduction: the lateral ventricles of the cerebral hemispheres are cover by cuboidal or cylindrical ependymal cells, which delimit the neuronal tissue of the ventricular lumen harboring cerebrospinal fluid. A high percentage of people with schizophrenia have a preferential enlargement of some segments of the ventricular system such as the temporal horn and the lateral ventricle body, these findings are one of the first neuro-structural changes found in this disease, especially by visualization with the use of simple magnetic resonance.

Materials and Methods: lateral ventricular dilations were analyzed by gross neuroanatomical findings of patients with schizophrenia and focused on the quantitative hypothetical study of a neurohistological variation that necessarily implies the ependymal cells of these ventricles.

Results: an increase in the total mass of ependymal cells lining the lateral ventricles of the brain can occur in the development of schizophrenia, associated with lateral ventricular dilatation due to an increase in the rate of cell division, corresponding to hyperplasia or histological adaptive variation during the neuropathological development of the ventricles by changing in the shape of a simple cylindrical epithelium or simple cubic epithelium to a simple plane epithelium and thus to cover the greater territory that involves the ventricular dilatation, finally, another possible option can be given by cellular migration from areas distant to the sector where cavitation of the lateral ventricle increases.

Conclusion: from the histological point of view, ependymal cells of the lateral ventricles must necessarily undergo neurohistological modifications in quantity, shape or adhesion in the ventricles of people with schizophrenia.

GLU-TUBULIN IS A MARKER FOR SCHWANN CELLS AND CAN DISTINGUISH BETWEEN SCHWANNOMAS AND NEUROFIBROMAS

Josune Garcia-Sanmartin[1], Susana Rubio-Mediavilla[2], José J. Sola-Gallego[2], Alfredo Martínez[1]

[1] Oncology Area, Center for Biomedical Research of La Rioja (CIBIR), Piqueras 98, 26006 Logroño, Spain
[2] Pathology Service, Hospital San Pedro, 26006 Logroño, Spain

Introduction: Schwann cells generate myelin sheaths around the axons of the peripheral nervous system, thus facilitating efficient nerve impulse propagation. Two main tumor types can arise from peripheral nerves, schwannomas and neurofibromas, which are sometimes difficult to distinguish and may require the use of diagnostic biomarkers. Here, we characterize a new marker for Schwann cells and its potential use as a diagnostic marker for schwannomas.

Material and methods: Immunohistochemistry for Glu-tubulin, a posttranslational modification of α-tubulin, was performed in mouse and human tissues. Primary cultures of fibroblasts and Schwann cells were established from mouse sciatic nerves, and Western blot analysis was performed. Clinical specimens of schwannomas (n = 20) and neurofibromas (n = 20) were stained with anti-Glu-tubulin antibodies.

Results: All peripheral nerves were immunoreactive for Glu-tubulin antibody, including large nerve trunks, thin myelinated nerves, as well as the myenteric and submucous plexus of the digestive tract. In the mouse brain, many neurons were immunoreactive for Glu-tubulin but oligodendrocytes were negative. During embryo development, immunoreactive nerves were already found at E10. In Schwann cells, the staining is restricted to the myelin sheaths and is not present in the perinuclear cytoplasm or the Ranvier nodes. Western blot analysis of primary cultures showed that Glu-tubulin immunoreactivity was found in the Schwann cells but not in the fibroblasts. Clinical specimens of schwannomas presented a strong staining in all tumor cells, whereas neurofibromas had a light speckled staining pattern, easily distinguishable from the one found in schwannomas.

Conclusions: Glu-tubulin can be used as a marker of Schwann cells and can help in diagnosing peripheral nerve tumors.

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SMALL MOLECULES RELATED TO ADRENOMEDULLIN REDUCE TUMOR BURDEN IN A MOUSE MODEL OF COLON CANCER.

Laura Ochoa-Callejero[1], Josune García-Sanmartín[1], Sonia Martínez-Herrero[1], Susana Rubio-Mediavilla[2] and Alfredo Martínez[1].

[1]*Angiogenesis Group, Oncology Area, CIBIR, Logroño, Spain.*

[2]*Pathology Service, Hospital San Pedro, Logroño, Spain.*

Introduction: Adrenomedullin (AM) expression is elevated in colon cancer patients and a correlation between higher AM levels and lower disease-free survival has been described. Nevertheless, the influence of AM in colon cancer is somewhat controversial. To investigate the contribution of AM and its gene-related peptide, proadrenomedullin N-terminal 20 peptide (PAMP), to the progression and potential treatment of colon cancer we have studied the effects of several small molecules (SM) related to AM and PAMP on a mouse model of colon cancer.

Material and methods: Four SM were tested: 16311 (a negative modulator of AM), 145425 (a positive modulator of AM), 87877 (a negative modulator of PAMP), and 106221 (a positive modulator of PAMP). For each SM, 4 experimental groups of male C57BL6 mice were used: i) Control group (injected with vehicle and drank regular water); ii) SM group (vehicle, regular water, and injected with the SM); iii) DSS group (injected with azoxymethane (AOM) and drank dextran sulfate sodium (DSS)); and iv) DSS+SM group (treated with AOM, DSS, and the SM).

Results: None of the mice belonging to groups i and ii developed any tumor or had any pathological finding, indicating that the SMs do not present overt toxicity. All mice in groups iii and iv developed colon neoplasias. No significant differences were found among mice treated with PAMP modulators indicating that PAMP may not play a major role in colon cancer or that the PAMP-related SMs were not very effective. On the other hand, mice that received 16311 had worse colitis symptoms than their control counterparts, whereas mice injected with 145425 had a lower number of tumors than their controls. SM 145425 regulated the expression of proliferation markers such as Lgr5 and it had also an impact on microbiota, preventing the DSS-elicited increase of the *Bacteroides/Prevotella* ratio.

Conclusions: These results suggest that AM may have a protective role during the progression phase of colon cancer, and that treatment with AM or with positive modulator SMs may represent a novel treatment for colon cancer.

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ORAL LICHEN PLANUS: AN ULTRASTRUCTURAL APPROACH AND CHARACTERIZATION OF TELOCYTES

Carmen Berga[1], Tomás Usón[2], Miguel Ángel Trigo[3], Javier Gracia-Llanes[1], Ignacio Javier Moral[2], Ramiro Álvarez[3], Tomás Castiella[4], Concepción Junquera[1].

[1] Human Anatomy and Histology Department, Faculty of Medicine, University of Zaragoza / Institute for Health Research Aragón (IIS), Zaragoza, Spain
[2] Maxillofacial Surgery Service, University Hospital Miguel Servet, Zaragoza
[3] Pathological Anatomy Service, University Hospital Miguel Servet, Zaragoza
[4] Pathological Anatomy Service, Faculty of Medicine, University of Zaragoza Zaragoza / Institute for Health Research Aragón (IIS), Spain

Introduction: Lichen Planus is a mucocutaneous, autoimmune and chronic disease whose etiology is unknown. It can affect skin and either oral or other types of mucosa. It shows different clinic forms from reticular lesions to atrophic-erosive shapes. Histopathological features are liquefaction degeneration of basal keratinocytes (hydropic degeneration), a band-like layer of chronic inflammatory cells and absence of epithelial dysplasia. Pathogenesis is an unknown complex process which ends up with the apoptosis of basal keratinocytes. Between the 0.4 – 5 % of lesions undergo to malignant conditions and what is more, malignant transformation still remains controversial. There is an absolute lack of studies about ultrastructure of Oral Lichen Planus (OLP) so the aim of our study is to make a broad description of ultrastructural characteristics.

Material and Methods: Three cases of OLP (reticular and erosive forms) were chosen among selected patients from the maxilofacial surgery department (University Hospital Miguel Servet). The samples were processed by Haematoxylin-Eosin (HE) technique and for electron microscope visualization. Ultrastructural examination was done with a transmission electron microscopy (TEM, Jeol 2020).

Results: Our research showed morphological changes in basal and spinous layer: a decrease of the number of cytoplasmic organelles in keratinocytes, several and prominent nucleolus, indented nucleus and presence of perinuclear vacuolization. The loss of desmosomes and consequent intercellular spaces are observed, and thus, keratinocytic cytoplasmic fragments containing desmosomal structures. Inside mitochondria we appreciate concentric lipidic forms which are similar to myelinic marks in residual bodies: these forms could be the result of oxidative stress in basal cells. This cellular damage, joint to hydropic degeneration could be responsible for keratinocytic apoptosis. Moreover, changes in basal lamina were observed and inflammatory cells were identified. We first describe the presence of telocytes in lamina propria.

Conclusions: Ultrastructural examination confers a relevant tool for approaching to understanding of the etiology of this pathology. The loss of cohesion at the beginning of malignant transformation and the relationship with telocytes may suggest the involvement of an epithelial-mesenchymal transition in OLP.

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ELECTRON MICROSCOPE EVALUATION OF IRREVERSIBLE ELECTROPORATION ON THE LIVER IN A PORCINE MODEL

Mª Concepción Junquera[1], Tomás Castiella[2], Javier Gracia-LLanes[1], Borja López-Alonso[3], Alba Hernáez[4], Lara García-Hernández[3], Antonio Güemes[4], Elaine Mejia[2], Pablo Iruzubieta[1], Alejandro Naval[3], Hector Sarnago[3], Oscar Lucia[3], Jose M. Burdio[3]

[1] Faculty of Medicine, Department of Human Anatomy and Histology, University of Zaragoza / Institute for Health Research Aragón (IIS), Domingo Miral s/n, 50009 Zaragoza, Spain.
[2] Faculty of Medicine, Department of Pathology, Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain / Institute for Health Research Aragón (IIS), Domingo Miral s/n, 50009 Zaragoza, Spain.
[3] Department of Electronic Engineering and Communications. Group of Power Electronics and Microelectronics
[4] Department of Surgery Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain Institute for Health Research Aragón (IIS), Domingo Miral s/n, 50009 Zaragoza, Spain.

Introduction: Irreversible electroporation (IRE) involves application of electric field pulses to cells leading to induced permanent nanopores on the plasma membrane providing a non-thermal cell death. The proposed mechanisms of apoptotic or necrotic cell death due to IRE include disruption of cell membrane caused by pore expansion and osmotic or chemical stress. In recent years, IRE has emerged as a new method of tumor ablation. In addition, IRE has been widely tested and per formed in several organs and tumors. Despite these promising developments, the fundamental mechanisms leading to cell death remain unknown. In this ultrastructural study, those modifications (from cell death to regeneration) occurring in different cellular types forming functional hepatic unit are assessed by using porcine livers under different experimental conditions.

Material and methods: In this study, 3 pigs (40 kg) were treated using parallel-plate electrodes separated by the width of the liver (i.e. 1-1.5 cm) and applying 100 pulses of 100 µs, with a frequency of 0.5 Hz to avoid thermal damage. Three electric field intensities were tested, one in each of the three used liver lobules: 1000 V/cm, 1500 V/cm, and 2 000 V/cm. Post-operative biopsies were performed at 3 h and 3 days after the stimulation. Samples were routinely processed for transmission and scanning electron microscopy visualization.

Results: Three hours after IRE application, alterations of cell membrane in the electroporated area were observed. They ranged from nanometric pores to wide disruption with spreading of organelles in the extracellular space. Cellular junctions between hepatocytes were not maintained. Dilated granular endoplasmic reticulum and mitochondria were observed. Autophagic processes initiated since presence of autophagolysosomes was evidenced. Surprisingly, Kupffer as well as Ito cells, erythrocytes and neutrophils showed conserved membranes. Fenestrated endothelia of Disse space also showed intact membranes. Three days after IRE application some hepatocytes showed nuclear fragmentation without disruption of cell membrane, suggesting apoptosis. However, the most of these hepatic cells showed necrosis, being then removed by macrophages. Subsequent changes demonstrating the beginning of regeneration of hepatocytes and *de novo* vascular formation were found.

Conclusion: Experimental data showed that ultrastructural study is an indispensable tool for reliable assessment of IRE ablation.

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HISTOTOXICITY OF THE ANTIRIBOSOMAL LECTIN EBULIN IN ELDERLY MICE

Manuel Garrosa[1], Pilar Jiménez, Damián Córdoba-Díaz[3], Verónica García-Recio[3], Sara Gayoso[1], Mª Ángeles Rojo[4], Manuel J. Gayoso[1], Tomás Girbés[2].

[1]Area of Histology. Faculty of Medicine and INCYL, University of Valladolid, Valladolid, Spain.
[2]Nutrition and Food Sciences. Faculty of Medicine, University of Valladolid, Valladolid, Spain.
[3]Pharmacy and Pharmaceutic Technology. Faculty of Pharmacy and IUFI, Complutense University of Madrid, Madrid, Spain.
[4]Department of Experimental Sciences. Miguel de Cervantes European University, Valladolid, Spain.

Introduction: Ebulin is a type-2 ribosome-inactivating protein (RIP) isolated by Girbés et al. (1993) from dwarf elder *(Sambucus ebulus L.)*, different isoforms being obtained from the different parts of the plant. Ebulin f has been isolated from the fruits, has an A and a B chain, the former shows glycosidase activity and the B chain displays capacity to bind to D-galactose, which confers it lectin character. Since the ancient times, fruits from the elders have been used as food and considered to have medicinal properties. However, they also may trigger toxic effects. We present a histological study of the toxicity of this protein throughout the body organs.

Material and Methods: A single dose of 5mg/Kg of water-dissolved ebulin f was i.p. administered to 12-month-old female Swiss mice, while litter mates were injected saline only. Two weeks after administration of the toxin, all surviving animals were anesthetized with isoflurane and sacrificed by decapitation. Samples of liver, heart, kidney, lung, spleen, adrenal gland, stomach, pancreas, small and large intestines, ovary, uterus and brain were taken and fixed by immersion in 4% paraformaldehyde. Pieces were processed for paraffin embedding, sectioned at 7 µm and stained for light microscopy with hematoxylin and eosin, Masson trichrome or TUNEL method. Care and manipulation of animals followed the European Community Council guidelines.

Results: Treated animals showed atrophy of Lieberkühn's crypts and subsequent derangement of the villi in the small intestine. Congestion of the lungs and pneumonic foci were also outstanding findings as well as centrolobulillar hepatic necrosis. Focal cardiomyocyte degeneration and scattered nephronal degeneration could also be found. TUNEL method revealed increased number of apoptosis in small intestine and liver. The rest of the organs did not show significant changes.

Conclusions: Elderly mice appeared more sensitive to ebulin administration than young animals, causing damage not only in the small and large intestines as formerly reported in these animals, but also in liver, kidneys, lung and heart. Caution must be taken when consuming unripe elderberries and on the other hand, the lower toxicity of ebulin regarding other RIPs like ricin, render it suitable for the construction of conjugates and immunotoxins to be used in targeted therapy against cancer.

INTEGRATED OPTICAL DENSITY ANALYSIS OF THE IMMUNOHISTOCHEMICAL EXPRESSION OF MART-1 IN HUMAN SKIN BIOPSIES WHIT MELANOCYTIC LESIONS.

Marco Paredes[1], Felipe Navarrete[1], Nelson Quilaqueo[1], Luciana Paredes[1].

[1]Departamento de Ciencias Básicas, Facultad de Medicina, Universidad de La Frontera, Temuco, Chile.

Introduction: The diagnosis of melanoma, in its initial stages, is problematic as it tends to be confused with melanocytic nevi, so new diagnostic strategies based on specific markers are used. A useful marker for the diagnosis of melanoma is MART-1. It is present in more than 85% of melanomas and is very useful to establish the diagnosis of metastatic melanomas.
Generally, the analysis of the immunohistochemical expression of this marker is of qualitative character, which generates errors in the interpretation of the data. Quantitative evaluation of the expression can be performed from the digital image through Integrated Optical Density (IOD) analysis. It is measured in pixels per area and allows the estimation of the relative amount of the object of interest relative to the background of the image. IOD, allows the evaluation of color differences in an image, pixel by pixel and convert it into numerical values to obtain a quantitative parameter.
The aim of this study was to evaluate DOI by the immunohistochemical expression of MART-1 in human skin skin biopsy sections diagnosed with melanocytic and nevus.

Material and Methods:
The analysis was performed from digital photographs obtained from histological sections of skin, incubated with MART-1 antibody and subsequently immunostained by the ABC-DAB system. IOD analysis was performed with Imagej Pro Plus 6.0 software (Media Cybernetic, USA). Samples of sixty-eight individuals were used.

Results:
The integrated optical density (IOD) analysis of skin biopsies allowed detecting differences in MART-1 immunohistochemical expression between nevus, melanoma and controls. Quantification by IOD showed an excellent correlation between MART-1 expression ($rs = 0.812$ $p < 0.001$) and pathological diagnosis.

Conclusions:
The results indicate that the IOD analysis of immunohistochemical expression of the MARK-1 melanoma marker can be quantified and used to differentiate nevi and melanoma in histological biopsies of human skin.

A NEW STAGING METHOD IN THE STUDY OF BONE DEFECTS CAUSED BY THE INFECTION WITH *STAPHYLOCOCCUS AUREUS*

Blanca Ibarra[1], Joaquin García[2], Galo Azuara[3], Blanca Vázquez [4], Ángel Asúnsolo[5], Miguel A Ortega[1], Natalio García-Honduvilla[1,6], Basilio De la Torre[7]; Julio San Román [4], Julia Buján[1].

[1]Departments of Medicine and Medical Specialities, Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares, Madrid, Spain. [2]Service of Orthopedic Surgery of University Hospital Principe de Asturias. [3]Service of Traumatology of University Hospital of Gudalajara. [4]Networking Biomedical Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain. [5]Department of Surgery, Medical and Social Sciences, Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares, Madrid, Spain. [6]University Center of Defense of Madrid (CUD-ACD), Madrid, Spain. [7]Director of traumatology service of University Hospital Ramón y Cajal.

Introduction. Joint prostheses are an essential element in the improvement of the quality of life. However, they may fail due to several factors, being the infection caused by *Staphylococcus aureus* one of the most frequent one. Looking for new fixing bone cements with less reactivity on bone tissue and an adequate response in case of infection is one of the main challenges today.

Material and methods. In this study, we evaluated the response of bone tissue in rabbits after introducing a hydroxyapatite coated titanium rod with a commercial fixative cement (Palacos®), compared to a new experimental cement containing PLGA microspheres. Both cements were tested in the presence and absence of *S. aureus* infection, as well as in the presence of different antibiotics. In order to evaluate the histological results, a new staging method is proposed. This method evaluates the degree of disruption of bone tissue from 0 to 10, allowing us to study the aggressiveness of the infection and the response of the infected tissue.

Results: The study showed a better behavior and a lower aggressiveness of the experimental cement with PLGA microspheres against the Palacos® commercial cement.

Conclusions: The application of the proposed new staging method allows us to take a step on the search of a gold standard which allows us to diagnose different degrees of bone destruction, both histologically and clinically.

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INTER AND INTRAOBSERVER VARIABILITY IN ASSESSING THE PROMINENCE OF THE NUCLEOLUS FOR GRADING RENAL CELL CARCINOMA

Marina Gándara-Cortés[1], José Antonio Ortiz-Rey[1], Débora Chantada[1], Begoña Iglesias[1], Laura Juaneda-Magdalena[1], Ángeles Peteiro[1], Pilar San Miguel[1], Cristina Fernández-Otero[2].

[1]Department of Pathology, Álvaro Cunqueiro University Hospital, Vigo, Spain
[2]Unidad de Metodología y Estadística. Instituto de Investigación Sanitaria Galicia Sur. Álvaro Cunqueiro University Hospital, Vigo, Spain

Introduction: The International Society of Urological Pathology has proposed a grading system for the renal cell carcinoma (RCC) based upon nucleolar prominence determined through microscopic examination of H-E slides. This four-stage system has been accepted by the WHO in 2016. We have analysed the inter and intraobserver reproducibility of this grading method.

Material and Methods: A total of 51 H-E sections from clear cell RCC and papillary renal cell carcinomas were selected, marking a 5 mm of diameter area on each slide for the microscopic evaluation. Six pathologists (4 staff members and 2 residents) were asked to assess the WHO 2016 grade (G1, G2, G3 or G4) in the marked areas. After 12 months they repeated the assessment (second run). Cohen´s Kappa analysis was performed following the Landis and Koch scale.

Results: The interobserver agreement in the first run was fair with a kappa= 0.381 [95%CI 0.265-0.497]. The six pathologists assigned the same grade in 17.6% of the cases (9 cases: 1 G1, 2 G2, 4 G3 y 2 G4) and five observers coincided in 27,5% (14 cases: 3 G1, 5 G2, 5 G3, 1 G4). The agreement did not improve in the second run (kappa= 0.297 [95%CI 0.191-0.402]). Grade 4 was the more reproducible of the four grades (good agreement: 0,613 in the first run, and 0,647 in the second run). With regard to the intraobserver agreement, it was good (0,667) for one observer, moderate for four observers including the two residents (0,458 to 0,540) and fair for a staff pathologist (0,355).

Conclusions: Although the new grading system for RCC was a simplification of the previous one (Fuhrman grade) we have found that a significant component of subjectivity persists causing problems of reproducibility among different observers. Taking account that the interobserver agreement did not improve in a second run and that intraobserver reproducibility has been better than the interobserver one, we think that the unsatisfactory agreement is more related to intrinsic difficulties of the subjective evaluation of this parameter than with the experience or the ability of the pathologist.

IMPORTANCE OF INFLAMMATION, OXIDATIVE STRESS AND HYPOXIA IN REMODELLING WALL IN PATIENTS WITH CHRONIC VENOUS INSUFFICIENCY: SPECIAL IMPLICATION IN INCOMPETENT VALVES

Miguel A Ortega[1], Javier Leal[1,2], Santiago Zubicoa[2], Beatriz Romero[1], María J Alvarez-Rocha[1], Blanca Ibarra[1], Melchor Alvarez-Mon[1,3], Julia Buján [1], Natalio García-Honduvilla[1,4]

[1]Departments of Medicine and Medical Specialities. Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares, Madrid, Spain. Networking Biomedical Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain.[2] Angiology and Vascular Surgery Service, Ruber International Hospital. Madrid, Spain. [3]Immune System Diseases-Rheumatology and Oncology Service, University Hospital Príncipe de Asturias, Alcalá de Henares, Madrid, Spain. [4]University Center of Defense of Madrid (CUD-ACD), Spain.

Introduction: Chronic venous insufficiency (CVI) mainly affects veins in the lower limbs. The affected vessels appear dilated, elongated and twisted, and the condition is often associated with incompetent valves (venous reflux). Our research focused on understanding whether hipoxia, inflammation and oxidative stress, which induce changes in the cytoarquitecture of vein walls, correlate with aging.

Patients and Methods: 110 patients were divided in two groups according to the diagnosis of the presence or absence of reflux. Patients were classified by age-younger vs older (under or over 50 years,respectively). Reflux studies were performed using noninvasive methods (7.5-10MHz color Doppler ultrasonography). Patients were classified as having no apparent clinical reflux (NR): Duration of venous reflux DVR<0.5 seconds (n=29),n=13 ≤ 50 years and n= 16 ≥ 50 years old. Patients with reflux (R) :DVR >0.5 seconds (n=81), n=32 ≤ 50 years and n= 49 ≥50 years old.The samples were processed for scanning microscopy, immunohistochemical and qRT-PCR analysis for inflammation (IL-6, MMP-2, MMP-9), oxidative stress (NOX1, NOX2), hypoxia (HIF-1α and HIF-2α), elastic and collagens fibers.

Results: Assessment of inflammation, oxidative stress and hipoxia markers showed a correlation with reflux in people under 50 years as well as in people over or equal 50 years without reflux. We also found that markers of inflammation and hypoxia increased in the venous walls of individuals with and without apparent clinical reflux, which indicates that the pathological changes occur prior to venous reflux. The inflammatory markers exhibited a significative increased in people under 50 years with reflux. Analysis of key vessel wall extracellular matrix component indicated that the synthesis of elastic fiber proteins and collagens increase in association with valve dysfunction and with aging

Conclusions: CVI is a multifactorial process that is a consequence of the progression of the venous pathology and is correlated with aging in people. In younger individuals, incompetent valves could be related to an asynchronous process of accelerated ageing.

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THE IMPORTANT ROLE OF PEDF FOR WALL CALCIFICATION AND RIGIDITY IN VENOUS REFLUX

Miguel A Ortega [1], Javier Leal [2], Santiago Zubicoa [2], Blanca Ibarra[1], Beatriz Romero [1], María J Álvarez-Rocha [1], Melchor Álvarez de Mon[1, 3], Julia Buján [1], Natalio García-Honduvilla [1,4].

[1]Departments of Medicine and Medical Specialities. Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares, Madrid, Spain. Networking Biomedical Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain.[2] Angiology and Vascular Surgery Service ,Ruber International Hospital. Madrid, Spain. [3]Immune System Diseases-Rheumatology and Oncology Service, University Hospital Príncipe de Asturias, Alcalá de Henares, Madrid, Spain. [4]University Center of Defense of Madrid (CUD-ACD), Spain.

Introduction: Venous reflux is a consequence of lower extremity venous hypertension. Metabolic changes and calcifications are present result. The present study attempts to understand the role different types of calcification, fibrinoid, and PEDF (serpins) play in this process.

Patients and Methods: This study included 110 patients with IVC classified by age (above or below 50 years of age) and the presence (n=61) or absence (n=29) of a diagnosis of valvular incompetence. Histopathologic studies for detection of calcium (Von Kossa) and fibrinoid (PTAH) were performed. The presence of serpins was demonstrated by immunohistochemical and RT-qPCR techniques.

Results: We observed an increase in metastatic calcification in patients depending upon age and pathology. However, dystrophic calcifications were noted in youner patients (below 50 years of age) with valvular incompetence. Venous wall calcifications and fibrinoid deposits correlate with an increase of serpin in patients with venous reflux.

Conclusions: PEDF function as well as fibrinoid contribute to an increase in the amount of calcium deposited in venous wall rigidity.

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EFFECTS OF LOW-LEVEL LASER ON THE TEMPOROMANDIBULAR JOINT IN THE RABBIT: MICROSCOPIC AND MACROSCOPIC ANALYSIS

Nilton Alves[1,2], Naira F. Deana[3].

[1]*Faculty of Dentistry, Universidad de La Frontera, Temuco, Chile.*
[2] *Applied Morphology Research Centre (CIMA), Universidad de La Frontera, Temuco, Chile*
[3] *Master Program in Dentistry, Faculty of Dentistry, Universidad de La Frontera, Temuco, Chile*

Introduction: Low-level laser (LLL) has bio-stimulant, anti-inflammatory and analgesic effects, and is an alternative for treating pathologies of the temporomandibular joint (TMJ). The animal model of choice for working with the TMJ is the rabbit, due to the structural and functional similarity of its TMJ with the human TMJ. The object of this study was to analyse the macroscopic and microscopic effects of different doses of LLL on the bone tissue and articular disc of the TMJ.

Material and Methods: We used 20 rabbits divided into 5 groups: 4 experimental (EG), 1 control (CG). Laser irradiation was carried out with *AsGaAl* 904nm, 2 application points, doses of: EG1-15J/cm^2, EG2-45J/cm^2, EG3-60J/cm^2, EG4-90J/cm^2. Both TMJs were dissected. The articular disc, mandibular fossa and mandibular condyle were measured with a digital calliper. Microscopic analysis was carried out with Masson's trichrome. The data were analysed with the IMAGE-J software. Statistical analysis was carried out with ANOVA and Tukey's test, with statistical significance determined by $p<0.05$.

Results: The thickness of the articular disc in the anterior and medial zones was augmented in EG2 (p=0.021 and p=0.019 respectively). In the posterior zone a greater augmentation in thickness was observed in the EG than the CG (p=0.000). There was an increase in the anteroposterior diameter of the mandibular condyle in groups EG2 and EG4 (p=0.004). An increase was observed in the depth of the mandibular fossa in EG1 (p=0.025). An increase was observed in the anteroposterior diameter of the mandibular fossa in the EG (p=0.011). Microscopic analysis showed alterations in the collagen fibres of the articular disc in the EG, with an increase in fibroblasts.

Conclusions: Laser irradiation produces alterations in the bone structures and the articular disc of the TMJ, the effects being dose-dependent. The alterations produced by very low or very high doses were less significant; a dose of 45J/cm^2 promoted the highest bio-stimulation of the bone and cartilage tissues, as well as an augmentation of the structures of the TMJ and an increase in fibroblasts.

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INDUCTION OF OSTEOARTHROSIS IN THE TEMPOROMANDIBULAR JOINT IN RABBITS: ANALYSIS BY CONE-BEAM COMPUTERISED TOMOGRAPHY

Nilton Alves[1,2], Naira F. Deana[3], Ivonne Garay[2]

[1]Applied Morphology Research Centre (CIMA), Universidad de La Frontera, Temuco, Chile.
[2] Faculty of Dentistry, Universidad de La Frontera, Temuco, Chile.
[3]Máster Program in Dentistry, Universidad de La Frontera, Temuco, Chile.

Introduction: Osteoarthrosis (OA) is a progressive pathology which involves cartilage degradation, remodelling of the subchondral bone, synovitis and chronic pain. The animal model of choice for working with the temporomandibular joint (TMJ) is the rabbit, due to the structural and functional similarity of its TMJ with the human TMJ. Cone-beam computerised tomography (CBCT) is the preferred imaging technique for assessing the osseous components of the temporomandibular joint (TMJ), providing essential information for the diagnosis of OA. The object of this study was to analyse imaginological changes visible by CBCT after the induction of OA in the TMJ of rabbits by monoidoacetate (MIA) and papain.

Material and Methods: We used 23 rabbits in total, 5 for the control group (CG) and 18 divided between two experimental groups, MIA and papain. Each experimental group was divided into 3 by date of euthanasia (15, 25 and 45 days); OA was induced by intra-articular injection. The doses used were 3 mg of MIA dissolved in 50 ml saline solution, and 0.3 ml of papain solution 1.6% split into 3 doses at days 1, 4 and 7. CBCT was carried out prior to euthanasia. Student's t-test and ANOVA were used for statistical analysis.

Results: The experimental group presented lower mean values than the CG, whether OA was induced by MIA or papain. On the surface of the condyles with OA induced by papain and MIA we observed: loss of the rounded form of the condyle, deformity of the condyle or mandibular fossa, cortical irregularity, cortical wear, increase in the vertical dimension of the condyle. The changes were more expressive in TMJ with OA induced by MIA. **Conclusions:** OA induction by MIA and papain generates changes observable by CBCT in the dimensions of the mandibular condyle in rabbits. Both inducers promote signs compatible with OA on the articular surfaces of the TMJ; MIA promotes more expressive changes.

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MICROANALYTICAL IONIC PROFILE OF OSTEOPOROTIC PATIENTS CANCELLOUS BONE

Crespo PV[1], Sánchez Quevedo, MC[1], Crespo-Lora V[2], García JM[1], Cano JR[3], Cruz E[3], Guerado E[3]

[1] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[2] UCG Anatomía Patológica, Complejo Hospitalario de Jaén, Spain
[3] Division of Traumatology and Orthopedic Surgery, Hospital Costa del Sol, Marbella, Spain

Introduction: Previous studies demonstrated that the microanalytical composition of lamellar cancellous bone corresponding to osteoporotic patients was similar to that of non-osteoporotic individuals. The aim of this work is to increase the sample size of the study and correlate the microanalytical results with other variables related to the patients such as gender, age and seric levels of calcium, phosphorous and vitamin D.

Material and methods: 40 patients were included in the study. 20 of them were treated for hip fracture due to osteoporosis and 20 were subjected to hip replacement due to coxarthrosis. A cancellous bone biopsy was taken from the femoral head of each patient and blood levels of calcium, phosphorous and vitamin D were quantified. Each biopsy was fixed in liquid nitrogen, washed in 3% H2O2, air-dried, sputter-coated and examined in a FEI Quanta 200 scanning electron microscope with an EDAX microanalytical detector. Ten analyses were taken per specimen. The P/B method was used to measure the concentrations of calcium and phosphorus in each bone sample using microcrystalline salt as standards. Statistical comparison between both groups of patients was carried out by using the Mann-Whitney test.

Results: All spectra obtained in the bone samples showed significant peaks for calcium and phosphorous. Microanalytical quantitative analyses showed that normal bone had a calcium concentration of 28.38% of the total bone content, whereas osteoporotic bone had 27.68% of calcium. The concentrations of P were 11.36% for normal bone and 11.40% for osteoporotic bone. The Ca/P ratio was 2.29 for both the normal bone and 2.40 for the osteoporotic bone. Microanalytical differences for bone Ca, P and Ca/P ratio were non-significant for the comparison of osteoporotic and non-osteoporotic bone. No differences were detected regarding gender or age of the patients. Blood levels of Ca, P and vitamin D were significantly higher in non-osteoporotic patients as compared to patients with osteoporotic fractures.

Conclusions: Bone density and mineralization was similar for the patient population with osteoporotic hip fractures and non-osteoporotic patients with coxarthrosis.

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TUMOR VASCULAR STRUCTURES ARE IMPORTANT FOR SEGMENTAL CHROMOSOME ABERRATIONS IN CIRCULATING TUMOR DNA DETECTION

Esther Gamero-Sandemetrio [1], Ana P. Berbegall [1,2], Irene Tadeo [1], Victor Zúñiga [1], Samuel Navarro[1,2], Rosa Noguera[1,2]

[1]Department of Pathology, Medical School, University of Valencia/ INCLIVA. Valencia, Spain. [2]CIBER of Cancer (CIBERONC), Madrid, Spain.

Introduction: Circulating tumor DNA (ctDNA) is tumor-derived fragmented DNA in the bloodstream or lymphatic system. Because ctDNA may reflect the entire tumor genome, it has gained impact for its potential clinical utility. To detect intratumoral genetic heterogeneity in neuroblastoma (NB), our group is developing an aSNP study in NB patients' blood obtained at diagnosis. In order to improve the knowledge of the mechanism of ctDNA release and link the differences between solid tumor tissues and ctDNA with segmental chromosomal aberrations (SCA) profile, we analysed: 1) The histological characteristics of blood and lymphatic vessels and their proximity to high amounts of apoptotic and necrotic cells and 2) Number of infiltrating phagocytes in the tumor tissue.

Materials and methods: The presence of SCA in tissue and plasma samples from 15 NB patients was performed using OncoScan aSNP platform (Affymetrix). Digital analysis was performed on tissue microarrays (TMAs) stained with antibodies anti CD31, D2-40, CD68 and CD163. The slides were scanned with Panoramic MIDI (3D Histech) and analyzed with Angiopath® for the vascular system characterization and with Panoramic viewer for the infiltrating phagocytes evaluation.

Results: The ctDNA genomic profiles were matched in 11 samples with the profiles detected in the corresponding tumors. These cases had a high density of scattered and irregularly capillaries swarming around the tumor cells, with or without presence of small lymph vessels and with some necrotic areas infiltrated by low number of phagocytes. Three ctDNA genomic profile showed additional SCA correlating with tumor tissues with increased density of capillaries and sinusoidal-like blood and lymph vessels, mainly located in the periphery of the tumor nests; large necrotic areas with great infiltration of $CD68^+$ and $CD163^+$ cells and large caliber of lymph vessels were also found. In the last case, some SCA were not detected in ctDNA; the tumor tissue presented low blood vessels density without necrotic zones nor phagocytes infiltration.

Conclusions: Tumor vascular structure (organization, density and shape) in addition to the degree of necrosis and phagocyte infiltration, influence the effective ctDNA release. Heterogeneous SCA tumor profiles have been facilitated by these histological studies.

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LACK OF ADRENOMEDULLIN AGGRAVATES COLITIS SYMPTOMS IN MICE THROUGH MICROBIOTA CHANGES AND ALTERED EXPRESSION OF TOLL-LIKE RECEPTOR 4

Sonia Martínez-Herrero[1], Ignacio M. Larráyoz[2], Judit Narro-Íñiguez[1], María J Villanueva-Millán[3], Emma Recio-Fernández[3], Patricia Pérez-Matute[3], Susana Rubio-Mediavilla[4], José A. Oteo[3], Alfredo Martínez[1].

[1]Oncology Area, Ctr. for Biomedical Research of La Rioja (CIBIR), Logroño, Spain, [2]Biomarkers and Molecular Signaling, CIBIR, Logroño, Spain, [3]Infectious Diseases Department, CIBIR, Logroño, Spain, [4]Department of Pathological Anatomy, Hospital San Pedro, Logroño, Spain

Introduction: the link between intestinal inflammation and microbiota has become evident in the last decade. Adrenomedullin (AM) is a peptide whose administration has therapeutic action in colitis and inflammatory bowel disease. Since it also has antimicrobial activity, we decided to evaluate the influence of AM in colitis and microbiota composition using an inducible knockout (KO) model for AM.

Material & Methods: we have developed an inducible AM KO using Cre/loxP technology combined with the induction system mediated by doxycycline, tet-On. Microbiota composition was analyzed by massive sequencing. Colitis was induced in mice by administration of 10 mg/Kg azoxymethane followed by 2.5% dextran sulfate sodium (DSS) in the drinking water. Colitis was evaluated using a clinical symptoms index, histopathological analyses, and qRT-PCR for inflammatory, adhesion, and toll-like receptor 4 genes.

Results: abrogation of the *adm* gene in the whole body was confirmed by PCR and qRT-PCR. KO mice exhibit significant changes in colonic microbiota: higher proportion of *δ-Proteobacteria* class; of *Coriobacteriales* order; and other families was observed in KO feces. Meanwhile these mice had a lower proportion of beneficial bacteria, such as *Lactobacillus gasseri* and *Bifidobacterium choerinum*. TLR4 gene expression was higher ($p<0.05$) in KO animals. AM deficient mice treated with DSS exhibited a significantly worse colitis with profound weight loss, severe diarrhea, rectal bleeding, colonic inflammation, edema, presence of inflammatory infiltrates, crypt destruction, and higher levels of pro-inflammatory cytokines. No changes were observed in the expression levels of adhesion molecules.

Conclusions: we have shown that lack of AM leads to changes in gut microbiota population and in a worsening of colitis condition, suggesting that endogenous AM is a protective mediator in this pathology.

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DIFFERENTIATION OF MESENCHIMAL STEM CELLS IN BONE REPAIR BY THE Wnt/β-CATENIN SIGNALING PATHWAY. CROSSTALK BETWEEN Musashi-1, Runx2 AND Periostin.

Vicente Crespo-Lora[1,2], Clara Cándido-Corral[2], Miguel Padial Molina[3], Pablo Galindo Moreno[3], Pedro Hernández-Cortes[4], Francisco O´Valle Ravassa[2].

[1]UGC Pathology Complejo Hospitalario de Jaén, Spain.
[2]Department of Pathology. Biopathology and Regenerative Medicine Institute (IBIMER), School of Medicine.University of Granada. Granada, Spain.
[3] Spain Department of Oral Surgery and Implant Dentistry, School of Dentistry, University of Granada, Granada, Spain.
[4]Orthopedic Surgery Department of PTS Hospital University of Granada.

Introduction: Mesenchymal stem cells (MSCs) are cells capable of differentiating into cells of mesodermal origin. The Wnt /β-catenin signaling pathway controls the differentiation of progenitor cells into chondrocytes and osteoblasts. Musashi 1 (Msi1) is strongly expressed in MSCs. Runx2 is considered the driver gene in bone formation at the beginning of osteoblastic differentiation, and Periostin is involved in the early stages of bone repair. The aim of our study is to analyze the immunohistochemical and molecular expression of Msi1, Runx2, and Periostin in an experimental fracture model and confirm their involvement in bone repair through the Wnt/β-catenin signaling pathway.

Material and methods: 20 male Wistar rats weighing 250 g were used in a unilateral femoral diaphyseal fracture experimental model without osteosynthesis. The left hind limbs were taken as controls. Euthanasia was performed at fifteen days. Samples were embedded in paraffin and the histopathological, immunohistochemical and molecular study was performed for Msi1, Runx2, and Periostin. Statistical analysis was performed with the SPSS 20.0 program.

Results: There is a significant nuclear immunohistochemical expression in Msi1 and Runx2 fibrocartilage compared to controls (Table 1). The periostin shows intense interstitial positivity in fibrocartilage and in bone tissue of the fracture callus. A high positive correlation between the expression of Periostin and Msi1 in fibrocartilage osteoprogenitor cells (rho = 0.881), as well as between Periostin and Runx2 in the osteoprogenitor cells fibrocartilage (rho = 0.9326) and periosteum (rho = -0,782) ($p < 0.0001$, Spearman's test).

Table 1: Comparison of the nuclear immunohistochemical expression of Msi1, Runx2.

	Msi-1			Runx2		
	Fracture	Control	P	Fracture	Control	P
Osteocytes (cels./mm^2)	373.8±77.4	8.0±5.7	0.001	249.1±95.9	0.0±0.0	0.001
Osteoblasts (cels./mm^2)	512.9±131.7	48.3±53.4	0.001	393.3±146.1	80.6±29.6	0.002
Condrocytes (cels./mm^2)	544.3±158.3	0.0±0.0	0.001	610.2±182.1	0.0±0.0	0.001
MSCs (cels./mm^2)	383.0±173.5	0.0±0.0	0.001	270.6±105.9	212.9±80.7	0.190

Conclusions: Increased expression of Msi1 suggests the involvement of this protein in the mechanisms of regulation of proliferation, differentiation and bone regeneration. The correlation between the expression of Msi1, Runx2 and Periostin suggests a possible involvement of these 3 proteins in the bone repair process via the Wnt/β-catenin signaling pathway

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PROOF OF CONCEPT FOR A MINIMALLY INVASIVE TREATMENT OF THE GANGLION IN EXPERIMENTAL RAT MODEL.

Vicente Crespo-Lora[1,4], Mercedes Caba-Molina[2], Jose Aneiros-Fernández[2], Pascual Vicente Crespo Ferrer[3], Francisco O´Valle Ravassa[4], Pedro Hernández-Cortes[5].

[1]UGC Pathology Complejo Hospitalario Jaén, Spain.
[2]Intercenter provincial unit of Pathology University Hospital Granada, Spain.
[3]Department of histology, tissue engineering,group School of Medicina. University of Granada. Spain
[4]Department of Pathology and Biopathology and Regenerative Medicine Institute (IBIMER), School of Medicine. University of Granada. Spain.
[5]Orthopedic Surgery Department of PTS Hospital. University of Granada. Spain

I**ntroduction**: The ganglion is a prevalent lesion, pseudocystic, joint location of unknown etiology; surgical removal is the treatment of choice, with a recurrence rate of up to 40%. Surgical excision leads to increased health expenditure and waiting lists, as well as possible neurovascular complications. Sclerosing foams have proved useful as a minimally invasive treatment with a high success rate and few side effects in treatment of various degenerative diseases. We propose a proof of concept for experimental treatment with sclerosing foam in an experimental model of ganglion.

Material and methods: Eight 250-300 g male Wistar rats were used, two neocysts were created by inserting crystal spheres of 1 cm diameter in the dorsal region; at 4 months the spheres were removed, neocysts were filled with hyaluronic acid; After 5 days, 8 cysts were treated with a single intracystic injection of ethoxysclerol (5 mg / ml) and the remainder were taken as controls. 14 days after treatment with sclerosing foam, the cysts were embedded in paraffin for histopathological, morphometric and immunohistochemical study for Vimentin, PCNA, Hcaldesmón, β-Actin, Actin Muscle Specific, Musashi-1 and Ki-67.

Results: Of the 16 neo-cysts, 1 was not correctly identified, leaving a total of 15 cysts (8 treated and 7 controls). At the morphometric level, the sclerosing foam treatment presented fusion of the cyst walls in, 6 of 8 cysts (75%), compared to the controls, 2 of 7 cysts collapsed (28.57%). Sclerosing foam did not produce morphological damage to the surrounding tissues and statistically significantly reduced the diameter and the percentage of cystic lumen versus controls (Table 1). In turn the immunohistochemical study did not reveal statistically significant differences between both groups, except for PCNA.

Variable	Treated	Control	P value
Greater distance (µm)	63.75±79.70	154±132.281	0.125
Smallest distance (µm)	9.275±17.313	23.571±31.105	0.308
Total Cyst area (mm^2)	2.088±1.78	2.714±1.087	0.297
Light area (mm^2)	0.069±0.120	0.455±0.316	**0.010**
Percent light (%)	3,607±5,641	17,180±11,209	**0.011**
Cyst wall area (mm2)	2.019±1.375	2.259±1.024	0.676

Conclusions: Treatment with ethoxysclerol has been shown to induce sclerosis of the cysts without producing side effects, allowing postulated as possible future minimally invasive ganglion therapy.

Acknowledgements: research group of the Andalucian Government CTS#138

OPTICAL PROPERTIES OF BIOENGINEERED HUMAN SKIN IN THE VISIBLE RANGE

Ionescu AM[1], Miñan A[1], Cardona JC[1], Garzón I[2], Alaminos M[2], Pérez MM[1]

[1] Department of Optics, Faculty of Sciences, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[2] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA

Introduction: The knowledge of the behavior of light as it travels through the skin is a very important aspect for the success of the methods used for the diagnosis of cancerous or non-cancerous skin lesions. When the only treatment available of these lesions is skin transplant, the optical characterization of the sample used in the transplant is recommendable. Due to the shortage of donor skin to replace the excised tissues, the development of human skin substitutes by means of tissue engineering may offer numerous therapeutic alternatives to the use of autologous tissue grafts.

Material and methods: In this study, we have generated a novel model of bioengineered human skin substitute using human cells obtained from skin biopsies and fibrin-agarose biomaterials. Once the epithelial keratinocytes were isolated and expanded in culture, we used fibrin-agarose scaffolds for the development of a full-thickness human skin construct and we evaluated the optical properties of this human skin substitute after 1,2,3 and 4 weeks of development in culture. Inverse adding doubling method was used to find the scattering and absorption of the bioengineered human skin model using total reflection and total transmission measurements made with a single integrating sphere.

Results: The results show that epithelial cells play a major role in the optical behavior of the human skin model generated in this study, especially in the scattering values. The samples with the epithelium layer displayed higher scattering values than the ones without, for all times in culture, decreasing with increasing wavelength. Whereas absorption is concerned, slightly lower values of absorption where obtained for the samples with epithelium than the ones without, maintaining almost constant values. Nevertheless, both scattering and absorption coefficients values were in the values range of the native human skin.

Conclusions: According to our results, we can conclude that our novel human skin model could be used as an alternative to the autologous skin grafts, from an optical point of view.

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GENERATION AND IN VITRO CHARACTERIZATION OF HYALINE CARTILAGE-DERIVED CHONDROCYTE MICRO-AGGREGATES

Paes AB[1], Durand-Herrera D[1], Sánchez-López D[2], Vela-Romera A[1], Campos F[1], Vanfleteren J[3], Verplancke R[3], Carriel V[1]

[1] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[2] Division of Oral and Maxillofacial Surgery, University Hospital Complex of Granada, Spain
[3] Center for Microsystems Technology (CMST), Ghent University – IMEC, Belgium

Introduction: Hyaline cartilage is the main component of most articular surfaces, where it provides a highly adaptable surface for the normal function of joints. The goal of cartilage tissue engineering is to produce natural-like functional human cartilage that could be used to repair or replace damaged tissues. However, currently available cartilage models failed to efficiently reproduce the complex structure and functions of human cartilage. A recently described method allowing the generation of bioengineering tissues resembling the structure of native tissues is the generation of multicellular spheroids (MCS) or micro-aggregates by using a self-assembly process from suspended cells. The aim of this study is to generate novel MCS tissues constructed from human hyaline chondrocytes.

Material and methods: To elaborate these MCS, 250,000 human hyaline chondrocytes isolated from human cartilage biopsies were seeded in agarose chips generated from tailor-made PDMS molds. Micro-aggregates were allowed to form and develop in culture using two different culture media (expansion and chondrogenic media). Cell viability, proliferation and cell damage was determined by Live/Dead, WST-1 and DNA quantification tests, respectively in each culture condition. As controls, 2D chondrocyte cultures (monolayer) were used.

Results: Microscopic images showed that chondrocytes cultured with expansion and chondrogenic culture media begin to aggregate from day one of development, and tended to form stable micro-aggregates between day two and three. According to the viability assays, MCS remained viable during the whole culture period, although cells with the chondrogenic medium showed less percentage of cell death. Regardless, cell death appears in the first place within the 2D culture. Moreover, the WST-1 assay determined that cell proliferation in 3D culture is lower than in 2D culture, whilst the proliferation levels are higher with the expansion medium in both culture types.

Conclusions: Chondrocytes are able to form stable MCS, remaining viable through the entire culture period, although there is a preference for the chondrogenic medium in terms of viability. However, the expansion medium allowed cells to proliferate into MCS. Further histological and molecular studies are still needed to determine the functionality and the synthesis of essential extracellular matrix molecules.

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ENDOTHELIAL DIFFERENTIATION CAPABILITY OF HUMAN MSC FOR THE GENERATION OF PREVASCULARIZED ORAL MUCOSA SUBSTITUTES

Zapater A, Mateu-Sanz M, Durand-Herrera D, Jaimes-Parra BD, García-Martínez LA, Martín-Piedra MA, Sánchez-Quevedo MC, Garzón I

Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA

Introduction: Vascularization is one of the major limitations of tissue engineered organs, including the human oral mucosa. Survival of bioengineered oral mucosa after in vivo grafting depends on the rapid establishment of an adequate blood supply from host tissues, what may be promoted by the presence of vascular progenitors in the artificial stroma. In this study, we have evaluated the endothelial differentiation capability of four populations of human MSC derived from bone marrow (BMSC), adipose tissue (ADSC), dental pulp (DPSC) and umbilical cord Wharton's jelly (WJSC) to be used in oral tissue engineering protocols.

Material and methods: BMSC, ADSC, DPSC, WJSC were cultured and differentiated to the endothelial lineage by using two different conditioning culture media for 21 days: endothelial cell growth medium (dECGM) and enriched M199 medium (dEM199). Umbilical cord endothelial cells -HUVEC- were used as control. Cells were characterized for the MSC differentiation markers CD90 and CD73 by flow cytometry. Differentiation was analyzed by immunofluorescence for CD31, CD45, VWF, VEGF.

Results: All cells cultured with dEM199 show high expression for CD90 and CD73 by flow cytometry. Immunofluorescence showed that all cell types cultured with dEM199 obtained high positive signal for CD45 and VWF. DPSC cultured with dEM199 had the highest levels of CD31 and VEGF expression.

Conclusions: Our results suggests that BMSC, ADSC, DPSC and WJSC have endothelial differentiation potential, although the best candidates for the generation of prevascularized oral mucosa bioengineered stromas are DPSC.

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GENERATION OF PRIMARY CELL CULTURES OF ORAL MUCOSA KERATINOCYTES USING PHARMACEUTICAL-GRADE ENZYMES. A HISTOLOGICAL AND CELL VIABILITY EVALUATION

Zapater A, Mateu-Sanz M, Durand-Herrera D, Jaimes-Parra BD, Martín-Piedra MA, Paes AB, Sánchez-Quevedo MC, Garzón I

Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA

Introduction: Tissue-engineered oral mucosa equivalents have been successfully constructed using oral mucosa keratinocytes and fibroblasts. To obtain these cells, oral mucosa biopsies must be treated by enzymatic digestion. However, enzymatic treatments have to be standardized in the context of GMP laboratories. The aim of this study is to evaluate the capacity of different pharmaceutical-grade enzymes suitable for GMP laboratories to generate epithelial cell cultures for tissue engineering protocols.

Material and methods: Oral mucosa biopsies were obtained and digested with commercial pharmaceutical-grade 0.05% Trypsin, 0.05% Trypsin-EDTA, 0.25% Trypsin-EDTA, 0.5% Trypsin-EDTA, Accutase, Tryple select and Tryple select CTS (5 digestion cycles). After each cycle, cells were harvested and cultured and cell viability was evaluated by calcein AM-ethidium homodimer-1. Remaining oral mucosa biopsies were histologically analysed to determine the capability of each enzyme to detach the epithelium.

Results: The highest cell viability percentages corresponded to Accutase, 0.25% Trypsin-EDTA, 0.5% Trypsin-EDTA and 0.5% EDTA with 78.70%, 72.25%, 69.23% and 67.31% of viability, respectively. The lowest viability were found for 0.05% Trypsin, Tryple Select, 0.05% Trypsin-EDTA and Tryple Select CTS. A heterogenic population of keratinocytes and fibroblasts was found for the enzymes with the highest cell viability. Histological analysis showed that the use of 0.25% Trypsin-EDTA and 0.5% Trypsin-EDTA was able to completely detach all epithelial cells from the biopsy, where 0.5% EDTA partially separated the epithelium and Accutase was not able to remove any epithelial cells.

Conclusions: Although 0.5% Trypsin-EDTA or 0.5% EDTA showed usefulness for isolation of oral mucosa epithelial cells, our results suggest that the use of 0.25% Trypsin-EDTA should be preferentially used with high cell viability.

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GENERATION OF HETEROTYPICAL MODELS OF THE HUMAN URINARY MUCOSA USING UMBILICAL CORD WHARTON'S JELLY STEM CELLS

Jaimes-Parra BD[1], Garzón I[1], Martín-Piedra MA[1], Durand-Herrera D[1], Santisteban-Espejo A[2], Chato J[1], Quintero-Campos P[1], Campos A[1]

[1] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[2] Servicio de Hematología y Hemoterapia, Hospital Universitario Puerta del Mar de Cádiz

Introduction: The urinary mucosa can be affected by multiple congenital or acquired pathologies. In most of these cases, it is necessary to replace the damaged tissue in order to restore its normal function, and production of viable urinary mucosa by tissue engineering could contribute to treatment of these patients. In this work, we have developed a complete heterotypical human urinary mucosa by using umbilical cord Wharton's Jelly Stem Cells (WJSC) in order to determine the potential of WJSC to differentiate into urinary mucosa-like epithelial cells.

Material and methods: Heterotypic models of urinary mucosa were generated with fibrin-agarose biomaterials with stromal human urinary mucosa cells. Then, WJSC were used to generate a epithelial-like layer on top of the biomaterial, and were cultured ex vivo for 7 and 14 days. Once the heterotypic models were generated, three culture media were used to maintain the bioartificial tissue model: Amniomax medium (basal medium for WJSC culture), QC medium enriched with EGF (basal medium for epithelial culture) and conditioned medium (MC) obtained from native urinary mucosa epithelial cells cultured with QC medium. All bioengineered samples were compared with native urinary mucosa controls. Histological evaluation was performed by H&E staining and histochemistry for identification of ECM components by alcian blue, Schiff periodic acid and Gomori's reticulin. Immunohistochemical detection of ZO-1 was achieved to determine the epithelial differentiation profile.

Results: Our results suggest that the highest levels of epithelial and stromal differentiation were reached in the heterotypic model using specific epithelial medium (QC). The histological analysis revealed a well-stratified epithelium, especially in QC cultured samples, at 7 and 14 days. The analysis of ECM components revealed that the amount of non-fibrillar components was higher in comparison with fibrillar components and the expression of ZO-1 cell-junction proteins was higher in QC cultured samples.

Conclusions: Our results highlight the possibility of generating artificial urinary mucosa with similar histological patterns as native tissue by using WJSC as a source of cells with epithelial-like differentiation potential. Future in vivo experiments should be carried out to confirm the translational potential of heterotypical urinary mucosa models.

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EFFECT OF ADIPOSE TISSUE-DERIVED AUTOLOGOUS STEM CELL (ASC) IMPLANT ON POST-FRACTURE BONE REGENERATION IN RATS (*RATTUS NORVEGICUS).*

Carolina Smok Soto[1, 2], Mariana Rojas Rauco[2].

[1] *Institute of Research and Innovation in health. Faculty of Health Sciences, Central University of Chile, Santiago, Chile.*
[2] *Program of Anatomy and Developmental Biology, ICBM. Faculty of Medicine, University of Chile, Santiago, Chile.*

Introduction: Stem cells derived from adipose tissue (ASCs) are a breakthrough in relation to bone regenerative medicine, as they have the ability to self-renewal, differentiation and paracrine stimulation to various types of tissues including bone and cartilage. The hypothesis of this study considers that fractures treated with ASCs, reduces time and increases bone regeneration and vascularization, aiming histologically evaluate bone regeneration and vascularization in these fractures.

Material and Methods: 24 young male Sprague Dawley rats were used. The specimens were divided into two groups: Group A (treated) and group B (control). In both groups, the rats were euthanized at 11 and 21 days post-fracture.

Results: A statistically significant difference was observed in the number of newly formed trabeculae and vascular density in the treated group compared to the control group, but not in the number of osteogenic and resorptive cells.

Conclusions: ASCs treated rats have a higher angiogenic and improved bone regeneration, mainly due to the capability of synthesis of the components of the extracellular matrix of these cells, and by the production of angiogenic and growth factors.

LONG-TERM STUDY COMPARING DIFFERENT CYANOACRYLATE TISSUE ADHESIVES FOR PROSTHETIC FIXATION IN ABDOMINAL WALL REPAIR

Claudia Mesa-Ciller[1], Gemma Pascual[3], Marta Rodríguez[1], Bárbara Pérez-Köhler[1], Mar Fernández-Gutiérrez[2], Julio San Román[2], Juan M. Bellón[1].

[1] Department of Surgery, Medical and Social Sciences, Faculty of Medicine and Health Sciences, University of Alcalá, and Networking Biomedical Research Centre on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Alcalá de Henares, Madrid, Spain.
[2] Polymer Biomaterials Group, Polymer Science and Technology Institute-Consejo Superior de Investigaciones Científicas (ICTP-CSIC), and Networking Biomedical Research Centre on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain.
[3] Department of Medicine and Medical Specialities, Faculty of Medicine and Health Sciences, University of Alcalá and Networking Biomedical Research Centre on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Alcalá de Henares, Madrid, Spain.

Introduction: As an alternative to sutures, meshes used for hernia repair can be fixed using cyanoacrylate-based adhesives, thus preventing postoperative pain cause by nerve entrapment and improving patient comfort. The main problem with cyanoacrylates is their toxicity, that is being solved by increasing the length of the lateral alkyl chain.
This preclinical study compares the long-term behavior of 2 different chain length cyanoacrylates that are already used in hernia repair and another 2 new ones for this application.

Material and Methods: Partial abdominal wall defects (5x3 cm) were repaired using a Surgipro mesh in 18 New Zealand white rabbits, and 5 groups were established according to the mesh fixation method: sutures (control), Glubran 2 (n-butyl), Ifabond (n-hexyl), and the new cyanoacrylates, SafetySeal (n-butyl) and Evobond (n-octyl). Six months after surgery, implants were collected and used to analyze the biomechanical strength of the integrated mesh, host tissue response and tissue adhesive degradation.

Results: After six months, a good host tissue incorporation of the mesh was seen in all groups, with scar tissue infiltrating mesh pores and completely covering the mesh, and all 4 cyanoacrylate adhesives were still observed after this time. Glubran 2 and Ifabond adhesives had a greater quantity of macrophage response around filaments and surrounding the adhesive compared to sutures. Cell damage caused by adhesives was similar between the different groups, and only Glubran induced significantly more damage than sutures. Collagen I/III mRNA expression was significantly lower when the mesh was fixed with Ifabond than the other fixation materials, and no differences were observed in collagen protein levels except for slightly reduced collagen I deposition in Glubran and Ifabond groups, and of collagen III in the suture group. No differences were seen in mechanical strength between suture and all the different cyanoacrylates.

Conclusions: All the cyanoacrylates adhesives showed good long term behavior and tolerance irrespective of their chain length. Cyanoacrylate residues persisted at six months indicating their incomplete degradation. Biomechanical strengths were similar across the tissue adhesive groups and neither were differences with the sutures.

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BEHAVIOR OF NEW GENERATION RETICULAR MESHES WITH ANTIADHESIVE TREATMENTS FOR INTRAPERITONEAL REPAIR OF ABDOMINAL HERNIA

Claudia Mesa-Ciller[1], Gemma Pascual[2], Verónica Gómez-Gil[1], Bárbara Pérez-Köhler[1], Marta Rodríguez[1], Juan M. Bellón[1].

[1] Department of Surgery, Medical and Social Sciences. Faculty of Medicine and Health Sciences, University of Alcalá. Networking Research Centre on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN). Alcalá de Henares, Madrid, Spain.
[2] Department of Medicine and Medical Specialities. Faculty of Medicine and Health Sciences, University of Alcalá. Networking Research Centre on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN). Alcalá de Henares, Madrid, Spain.

Introduction: The intraperitoneal placement of a prosthetic mesh can lead to an inflammatory reaction into the abdominal cavity which induces the development of permanent adhesion between the mesh and the visceral peritoneum. Currently available types of mesh include reticular, laminar o composite prosthesis, but all of them reveal some disadvantages, and the ideal prosthesis for ventral hernia repair is not yet identified. The aim of this study is to evaluate the use of two-component monofilament mesh (Dynamesh) and a reticular polypropylene mesh covered with titanium (Timesh) in a preclinical model of intraperitoneal implant. Two conventional materials, a reticular polypropylene mesh (Surgipro) and a laminar polytetrafluoroethylene layer (Preclude) are used as control of adherence formation.

Material and Methods: Under general anaesthesia, mesh fragments of 5x3.5 cm were fixed using 6 transmural polypropylene stitches over the intact parietal peritoneum of 24 white New Zealand rabbits (n=6 per study group). During the postoperative period, the animals were subjected to a laparoscopy at 3, and 7 days, to follow the sequential adhesion formation process and changes occurring at peritoneal interface. All the animals were sacrificed 14 days after surgery, at which time tissue/prostheses specimens were obtained for light and scanning electron microscopy. The areas of the different implants covered by adhesions were estimated by image analysis. Results were compared using the Mann-Whitney U-test.

Results: While Preclude was free of adhesions, all the reticular prosthesis showed similar behaviour in terms of adhesion formation. Adherences were observed at 3 days postimplant and classified as firm, involved both the bowels and the omental tissue. No differences were observed in adhesions scores recorded at 3, 7 and 14 days postimplant for each mesh. Reticular prostheses were infiltrated by disorganized scar tissue with fibers concentric to the mesh filaments, while Preclude was encapsulated by organized tissue with fibers running parallel to their surface.

Conclusion: Our findings suggests that the use of different materials for reticular mesh designs does not seem to provide an advantage in terms of reduced adhesion formation at the peritoneal interface, so the biomaterial structure rather than their composition seems to be related with this process.

BIOMECHANICAL PROPERTIES OF A HUMAN SKIN SUBSTITUTE GENERATED BY TISSUE ENGINEERING

Durand-Herrera D, Campos F, Vela-Romera A, García-García O, Chato J, Martín-Piedra MA, Alaminos M, Garzón I

Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA

Introduction: Human skin is the outer layer of the human body and its functions are protection, sensitive response, heat regulation, control of evaporation, storage, synthesis, excretion and absorption. To fulfill these functions, human skin should have specific physical characteristics such as elasticity and stress resistance. Several bioengineered skin models have been designed. However, a low amount of studies focused on the study of the physical properties of these models. The objective of this study is to evaluate the histological and mechanical properties of an artificial skin model created by tissue engineering.

Material and methods: First, a skin dermis substitute was generated by using fibrin-agarose biomaterials with human skin fibroblasts immersed within. Then, a full-thickness human skin substitute was generated by culturing human skin keratinocytes on top of the dermis substitute. Both the dermal and the full-thickness substitutes were analyzed after 7 and 28 days of in vitro development. For histological analysis, all samples were fixed and embedded in paraffin to obtain 5um histological sections that were stained with hematoxylin and eosin. To evaluate the biomechanical properties of both models generated by tissue engineering, tissues were analyzed to determine their stiffness and deformability under tensile tests by determining the Young's modulus, stress at break and strain at break.

Results: The histological analysis revealed that fibrin-agarose skin appropriately supported growth and development of the stromal fibroblasts and the generation of an epithelium with up to cell 3 layers on top. The tensile tests analysis showed that the Young's modulus tended to increase over time ($p<0.05$), but the stress and strain at break did not vary. Statistically significant differences were observed between the artificial dermis and the full-thickness artificial skin at 28 days ($p<0.05$).

Conclusions: These results suggest that the presence of an epithelial layer on top of the dermal substitute is able to improve the biomechanical properties of the bioengineered skin. Therefore, the complete artificial skin could resist higher tensile forces than the dermal substitutes.

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GENERATION AND CHARACTERIZATION OF WHARTON'S JELLY STEM CELL MICROTISSUES FOR TISSUE ENGINEERING

Durand-Herrera D[1], Campos F[1], Quintero-Campos P[1], Fernández-Valadés R[2], Sánchez-Quevedo MC[1], Campos A[1], Alaminos M[1], Carriel V[1]

[1] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[2] Division of Pediatric Surgery, University Hospital Complex of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA

Introduction: Tissue engineering has shown promising results in generating tridimensional substitutes. Generation of 3D structures using microtissue technique has shown to increase function and cellular differentiation, and these structures could be used to fabricate highly functional and biomimetic tissue substitutes. The objective in this study is to generate and characterize microtissues using Wharton's jelly stem cells (WJSC) for their application in tissue engineering.

Material and methods: Human WJSC were isolated from umbilical cords and expanded until passage 5. In order to generate microspheres, 50,000 cells were seeded on agarose chips containing 256 micro-wells with an average size of 400x800 μm. The microsphere formation was controlled daily by phase contrast microscopy. The cell viability (as determined by live/dead, WST-1 and DNA release methods), structure and histological properties of these microspheres were analyzed at 4, 7, 14, 21, 28 days of ex vivo culture.

Results: WJSC were able to self-assemble and form microspheres from day 4 onward, and cells adopted a clearly peripheral organization with a capsule-like structure with an eosinophilic ECM-rich core from day 7. These microspheres synthetized high amounts of proteoglycans at days 4 and 7, which was restricted to the periphery at days 14 and 28. Histochemistry and immunohistochemistry revealed positive expression of collagens, especially among cells and at the core of the microspheres. Type III collagen was strongly positive in the core, whilst type IV collagen was found between the core and the peripheral cells. The WJSC lineage was confirmed by a strong positive reaction for CK AE1/AE3 and CK8.

Conclusions: This study demonstrated that WJSC is a promising cell source for the generation of microspheres for stromal tissue regeneration. WJSC generated morphologically stable microspheres with abundant extracellular matrix. Future in vivo studies are necessary to elucidate the potential clinical use of these microspheres or their ECM in skin, oral mucosa, cornea, nerve and palate tissue engineering.

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RHEOLOGICAL CHARACTERIZATION OF CHEMICALLY CROSS-LINKED FIBRIN-AGAROSE BIOMATERIALS FOR TISSUE ENGINEERING

Campos F[1], García-García O[1], Vela-Romera A[1], Crespo PV[1], Sánchez-Montesinos I[2], Garzón I[1], Sánchez-Quevedo MC[1], Carriel V[1]

[1] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[2] Department of Human Anatomy and Embryology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA

Introduction: The generation of biomaterials with adequate biomechanical and structural properties is still a challenge in tissue engineering. Hydrogels, the most used biomaterials, are highly biocompatible, although, in general, they do not have an optimal biomechanical behavior for tissue engineering purposes. Glutaraldehyde and genipin has been used as cross-linker agents to improve the structural and biomechanical properties of different kind of hydrogels. The aim of this study was to compare the effects of both types of cross-linker agents in native and nanostructured fibrin-agarose hydrogels (FAH and NFAH, respectively), a biomaterial which has proved to be very useful for tissue engineering protocols.

Material and methods: In this study, FAH and NFAH were elaborated by following previously described protocols (1,2). The constructs were subjected to chemical cross-linking with 0.25% glutaraldehyde and genipin. Hydrogel porosity was analyzed by scanning electron microscopy. The biomechanical properties of cross-linked and control hydrogels were determined by rheological analysis using a Bohlin rheometer. Mann-withney test was used for statistical comparisons.

Results: Our results show that chemical cross-linking did not modify the porosity pattern and fiber density of FAH and NFAH, suggesting that the capability of the biomaterial to host viable cells was not affected. Rheological analysis showed that the values of rigidity, elastic and viscous moduli were significantly improved with the use of glutaraldehyde and genipin cross-linking. However, these moduli were significantly higher when glutaraldehyde was used as compared to genipin ($p<0.05$).

Conclusions: These results suggest that the use of chemical cross-liniking agents improve the biomechanical properties of FAH and NFAH without affecting the structural porosity pattern associated to cell attachment.

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SURGICAL REPAIR OF SCLERAL DEFECTS BY USING ACELLULAR NANOSTRUCTURED FIBRIN-AGAROSE HYDROGELS

Sánchez-Montesinos I[1], Durand-Herrera D[1], González-Gallardo C[2], Campos F[3], Chato J[3], Vizcaíno G[3,4], Garzón I[3], Sánchez-Quevedo MC[3], Carriel V[3]

[1] Department of Human Anatomy and Embryology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[2] Division of Ophthalmology, University Hospital Complex of Granada, Spain
[3] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[4] Universidad Autónoma de Santo Domingo, República Dominicana

Introduction: The sclera is the outer connective tissue layer of the eye. This layer provides the structural support and shape to this organ, the resistance to the internal ocular pressure and it is the site of insertion of the ocular muscle. Despite its resistance, the sclera could be affected by traumatic injuries and cancer that could create structural defects with limited regeneration capability. These defects are often repaired by using cadaveric scleral allografts with variable results. This is a donor-dependent method that could transmit infectious agents and there is risk of tissue rejection. In this study, the aim was to explore the potential clinical usefulness of acellular nanostructured fibrin-agarose hydrogels (NFAH) as grafts to repair scleral defects.

Material and methods: A 5 mm diameter scleral defect was generated in 9 adult New Zealand laboratory rabbits under general anesthesia. Defects were surgically repaired with 5 mm diameter grafts of NFAH. After 35 days, animals were euthanatized and the eyes were processed for histological and histochemical analyses.

Results: Clinically, all defects were successfully repaired with NFAH with good macroscopic and clinical integration and with no signs of rejection, infection or other complications. Histological and immunohistochemical analyses carried out with picrosirius and alcian blue confirmed the absence of rejection or inflammation associated to the implants and confirmed the presence of a newly-formed extracellular matrix (ECM) in the repaired defects with some macrophages. This new tissue showed similar tissue organization and ECM density of normal sclera. Grafted tissues were found completely immersed into the scleral wall without signs of ECM integration.

Conclusions: This in vivo preclinical study demonstrated for the first time the possibility to repair the sclera by using only acellular biomaterials. Histology confirmed tissue regeneration in the experimental group, which was characterized by the presence of a newly formed scleral wall. However, further time-course studies are needed in order to determine the time required to reach complete regeneration.

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IN VIVO EVALUATION OF BIOARTIFICIAL HUMAN CORNEAS GENERATED WITH ALTERNATIVE CELL SOURCES

Garzón I[1], Martín-Piedra MA[1], González-Gallardo C[2], González-Andrades M[3], Muñoz-Ávila JI[2], Medialdea S[2], García-García O[1], Alaminos M[1]

[1] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[2] Division of Ophthalmology, University Hospital Complex of Granada, Spain
[3] Cornea Unit, Ophthalmology Department, Harvard University, Boston, MA, USA

Introduction: The efficient generation of bioartificial human anterior corneas recently allowed the treatment of severe corneal diseases. However, construction of bioengineered anterior corneas depends on the availability of human cornea biopsies used for the isolation and expansion of epithelial and stromal cells requiring long culture times. One of the possible alternatives to this technique is the use of mesenchymal stem cells (MSC). MSC demonstrated to have high proliferation and differentiation capabilities, including the epithelial lineage. In the present work, we have fabricated human anterior artificial corneas using alternative cell sources for the epithelial layer.

Material and methods: Primary cultures of human cornea epithelial and stromal cells were generated from cornea-limbal biopsies using explant and enzymatic digestion methods. Then, two types of bioengineered anterior human corneas were generated with nanostructured fibrin-agarose biomaterials: orthotypic corneas with epithelial corneal cells and heterotypic corneas with MSC on top of the artificial stroma. Both cornea models were evaluated in vivo by anterior keratoplasty in laboratory rabbits. Results were evaluated clinically and histologically after 12 months of the surgical procedure.

Results: Our results showed that both cornea models properly integrated in the host cornea. After 12 months, the biomaterial was remodeled and the histological structure of the graft stroma and epithelium was similar to the rabbit cornea, although the implant site was still evident at this time. As compared to orthotypic corneas, heterotypic artificial tissues were associated to a lower degree of cornea neovascularization.

Conclusions: These results suggest that MSC can be used for the efficient generation of human anterior corneas by tissue engineering, and these corneas may have in vivo usefulness in laboratory animals.

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DESIGN OF A MORPHOSHAPE BIOREACTOR FOR THE GENERATION OF BIOARTIFICIAL HUMAN CORNEAS WITH CONTROLLED SHAPE AND CURVATURE

Garzón I[1], Cardona JC[2], Ghinea R[2], Ionescu A[2], González-Andrades M[3], Muñoz-Ávila JI[4], Medialdea S[4], Alaminos M[1]

[1] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[2] Department of Optics, Faculty of Sciences, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[3] Schepens Eye Research Institute and Massachusetts Eye and Ear, Department of Ophthalmology, Harvard Medical School, Boston, Massachusetts, USA
[4] Division of Ophthalmology, University Hospital Complex of Granada, Spain

Introduction: Morphology is one of the most important factors affecting the refractive power of the human cornea, and changes in the shape and curvature may be associated to visual impairment. Several models of bioengineered human corneas have been generated in the laboratory using natural biomaterials such as fibrin and agarose. However, most biocompatible hydrogels have poor biomechanical properties and do not allow the generation of bioartificial corneas whose morphology resembles the native cornea. In the present work, we describe a novel method allowing the efficient generation of a human cornea with adequate morphology by using a specific bioreactor.

Material and methods: First, we generated a bioartificial human cornea model using primary cultures of cornea cells and fibrin-agarose biomaterials. Then, these corneas were nanostructured by plastic compression by using a specific bioreactor called Morphoshape that was designed in the laboratory and fabricated in a 3D printer. Briefly, this bioreactor consisted of a core with a guide in which a module with the morphology of the anterior human cornea is set. Then, a piston containing a complementary module with the shape and curvature of the posterior human cornea is set on top of the core. The bioengineered cornea is subjected to controlled pressure between both modules of the Morphoshape while most water is extruded from the biomaterial. These corneas were analyzed histologically.

Results: As compared to control artificial corneas, Morphoshape corneas displayed a curve shape resembling the human cornea. Morphology was not modified over time in culture, although corneas tended to swell and retain some liquid ex vivo.

Conclusions: The use of a specific Morphoshape bioreactor allowed the efficient generation of artificial corneas with the morphology of the human cornea, suggesting that their refractive power could be similar to that of the native cornea.

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NOVEL CHEMICALLY DECELLULARIZED PERIPHERAL NERVES ALLOGRAFT SUPPORT AXONAL REGENERATION AND MYELINATION IN VIVO

Chato J[1], Philips C[2], Durand-Herrera D[1], García-García O[1], Martín-Piedra MA[1], Campos A[1], Cornelissen M[2], Carriel V[1]

[1] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[2] Department of Basic Medical Sciences (Tissue Engineering Group), Ghent University, Belgium

Introduction: Critical nerve gaps are commonly bridged by the use of autologous nerves, which is the current gold-standard technique. However, there are well-known disadvantages associated to this method and novel alternatives are in need. Decellularization tissue engineering techniques make possible the generation of acellular scaffolds for regenerative purposes. In this context, the aim of this study was to evaluate a novel Roosens decellularization protocol (1) for the generation of decellularized nerve allografts (DN). These DN were used to repair 10-mm nerve gap in rats, compared to other previously described DN (2) and autograft. Peripheral nerve regeneration was determined by histology and functional tests.

Material and methods: In this study 24 Wistar rats were anaesthetized and 10-mm sciatic nerve defect was created and repaired by using Roosens, previously described DN and autograft as controls (n=6 each). After 12 weeks rats were subjected to clinical functional test. Furthermore, the implanted grafts were harvested, fixed, embedded in paraffin and sections were stained with MCOLL histochemical method and immunohistochemistry for GAP-43 and S-100.

Results: Clinical analyses did not show significant differences among the three groups, although previously described DN showed better motor and sensory recovery. Histology confirmed peripheral nerve regeneration in all groups, which was composed by a variable amount of newly-formed nerve fascicles. MCOLL technique demonstrated high levels of remyelination in autograft as compared to DN. Between both DN, results were comparable, although previously described DN showed slightly better results.

Conclusions: This comparative in vivo study demonstrated the regenerative potential of DN generated by using the Roosens method for tissue decellularization. Regeneration profile was comparable to previously described DN, but nerve autograft remained as the best substitute in terms of regeneration. Further studies are needed to improve the regenerative and biological properties of these novels DN.

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DISC SURFACE ROUGHNESS AND MELATONIN ADDITION INFLUENCE ON MORPHOLOGICAL PROPERTIES OF MG63 CELLS GROWN ON TITANIUM DISCS

José Javier Martín de Llano[1], Carolina Pérez-Martínez[2], Manuel Mata[1,3], Lara Milián[1], María Oliver[1], Carlos Labaig-Rueda[2], Antonio Fons-Font[2], Carmen Carda[1,4], M. Fernanda Solá-Ruiz[2].

[1]Department of Pathology, Faculty of Medicine and Dentistry, University of Valencia, and Health Research Institute of the Hospital Clínico (INCLIVA), Valencia, Spain.
[2]Department of Stomatology, Faculty of Medicine and Dentistry, University of Valencia, Valencia, Spain.
[3]CIBERES, Instituto de Salud Carlos III, Spain.
[4]Ciber-BBN, Instituto de Salud Carlos III, Spain.

Introduction: Osseointegration of titanium implants depends on several factors, as the physico-chemical properties of the metallic surface. Bioactive molecules could improve the osseointegration process by promoting undifferentiated cells to develop an osteoblastic phenotypen. We are studying the effects that the implant surface and melatonin have on the properties of MG63, an osteoblastic-like human cell line. Herein we describe the effects of those factors on morphological characteristics of these cells.

Material and Methods: Three groups of grade V titanium alloy (Ti6Al4V) discs were used: machined discs (M-group), discs which surface was acid etched (E) and discs E to which surface calcium phosphate particles were added (EP). MG63 cells were seeded on them and cultured in DMEM media. 24 and 72 h after cell seeding in culture media containing or not 50 μM melatonin discs were fixed and stained with eosin and DAPI. Images of the disc surface were recorded with a fluorescence microscope and a digital camera. Cell morphological parameters of 500 cells from each experimental group were calculated by using Image J program.

Results: Surface roughness was lower in M-group compared to E- and EP-group discs. Cell projection area (CPA) values (mean ± SD) for discs from groups M, E and EP were 2.421 ± 807, 1.973 ± 624 and 1.688 ± 740 μm^2, respectively after 24 h cell culture and 2.059 ± 610, 1.889 ± 634 and 1.660 ± 524 μm^2 after 72 h cell culture. There were statistically significant differences between every pair of disc groups compared, both at 24 and 72 h cell culture. For each group CPA was higher at 24 than at 72 h cell culture. In the presence of melatonin a lower CPA was obtained in all the experimental conditions but similar results and differences between every pair of disc groups were obtained.

Conclusions: Implant surface modified cell morphology, as cells growing on a rough surface had a lower CPA than those growing on a flatter surface. Melatonin had a similar effect, as cells grown in its presence had a lower CPA. We will discuss the influence of these variables on osteoblastic differentiation.

TISSUE RESPONSE TO TITANIUM IMPLANTS COATED WITH A HYBRID SILICA SOL-GEL IN A RABBIT TIBIA MODEL

José Javier Martín de Llano[1], Nuno Araújo-Gomes[2], Francisco Romero-Gavilán[2], Ana María Sánchez-Pérez[2], Mikel Azkargorta[3], Felix Elortza[3], Mariló Gurruchaga[4], Isabel Goñi[4], Rubén Salvador-Clavell[1], Lara Milián[1], Julio Suay[2], Carmen Carda[1,5].

[1]Department of Pathology, Faculty of Medicine and Dentistry, University of Valencia, and Health Research Institute of the Hospital Clínico (INCLIVA), Valencia, Spain.
[2]Department of Engineering of Industrial Systems and Design & Department of Medicine, University Jaume I, Av. Vicent-Sos Baynat s/n, Castellón, Spain.
[3]Proteomics Platform, CIC bioGUNE, CIBERehd, ProteoRed-ISCIII, Bizkaia Science and Technology Park, Derio, Spain.
[4]Chemical Science Faculty. Basque Country University, P. M. de Lardizábal 3, San Sebastián, Spain.
[5]Ciber-BBN, Instituto de Salud Carlos III, Spain.

Introduction: Superficial treatments of titanium dental implants modify their physico-chemical properties and eventually could improve the post-implantation healing process. We are analyzing the effect of a silica hybrid sol-gel coating on the biological response from a molecular, cellular and tissue level. Herein we describe the short-term tissue response to uncoated and silica coated implants.

Material and Methods: Titanium grade 4 uncoated dental implants (SAE-Ti), and implants coated with a 35:35:30 formulation of methyltrimethoxysilane:3-(glycidoxypropyl)-trimethoxysilane: tetraethylorthosilicate (35M35G30T) were surgically implanted in the tibia of rabbits. Two weeks post-surgery animals were euthanized and the implant and surrounding bone was embedded in poly(methyl methacrylate). A slice was obtained and stained with Stevenel's blue and van Gieson's picro-fuchsin. Images of the samples were recorded with a microscope and a digital camera. Bone-implant contact (BIC) in the cortical region and the length of osteoclast-like and foreign body giant cells contacting the implant or coating surface in the bone medullar cavity were morphometrically evaluated.

Results: Osseointegration of SAE-Ti titanium implants did not show statistically significant differences from that of 35M35G30T implants (BIC values 51.35 ± 15.29 % and 33.67 ± 22.34 %, mean ± SD, respectively). Along the medullary cavity, at the implant- or coating-tissue interface, was observed connective tissue, inflammatory components and two types of multinucleated cells; one type similar to osteoclasts and other resembling foreign body giant cells. Cells smaller than 100 μm were considered as osteoclast-like and the larger ones were classified as giant cells. Osteoclastic cells from SAE-Ti and 35M35G30T implants had a similar length (44.4 ± 21.6 μm and 40.4 ± 27.9 μm, respectively). Giant cells were smaller in the SAE-Ti group compared to the 35M35G30T group (162.0 ± 73.6 μm and 268.0 ± 140.0 μm, respectively).

Conclusions: The coating formulation did not interfere in the bone repair process but induced a significant increase of foreign body giant cells on implant regions distant from bone tissue. This might favor the degradation of the coating and afterward normal tissue development contacting the implant. Factors favoring implant osseointegration could be added to silica sol-gel coating formulations allowing a controlled release of them.

STUDY OF THE DERMAL COMPONENT ON A LONG-TERM HUMAN SKIN MODEL AND CHANGES ASSOCIATED WITH PRESSSURE ULCERS

Miguel A. Ortega[1], Lara Cristóbal[1,2], Beatriz Romero[1], Natalio García-Honduvilla[1,3], Julia Buján[1], Andrés A. Maldonado[1,4].

[1]Departments of Medicine and Medical Specialities, Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares. Networking Biomedical Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain. [2]Department of Plastic Surgery, La Zarzuela Hospital, Madrid, Spain.[3]University Center of Defense of Madrid (CUD-ACD), Spain.[4]Department of Plastic, Hand and Reconstructive Surgery, BG Unfallklinik Frankfurt, Frankfurt, Germany.

Introduction: Regenerative medicine and tissue engineering have made attempts to recreate human skin, but reproducing its complexity with skin substitutes remains a goal to achieve. Autografts are the gold standard in the clinical scenario, however, long-term changes in dermis, as well as its response after injuries, are not well known. This study analyzes the dermal scaffold and extracellular fibrillar protein changes on a long-term human skin model grafted onto a mouse before and after a mechanical pressure.

Material and Methods: Immunosuppressed NOD/Scid mice (n=10) were engrafted with human skin of dimensions 4x3 cm. After 60 days, a pressure ulcer (PU) was created in the permanent human skin using a compression device. Three study groups were established: native human skin, full-thickness skin graft before (hFTSG) and after applying mechanical pressure (hFTSG-PU). Evaluations were conducted with visual and histological assessment. The extracellular matrix protein profiles of samples from each group were compared by immunohistochemical staining for tropoelastin, collagen I and III, fibulins, and lysil oxidases (LOX) among others.

Results: The long-term engrafted skin (hFTSG) showed active expression of MMPs and tropoelastin compared to native skin. A reparative response after PU was observed: a statistically significant increase of fibrillin microfibrils components (TGF−β, MAGP-1 and fibrillin-1), and matrix components of collagen I, III and LOX were observed in dermal tissue of the hFTSG-PU group.

Conclusions: Our human skin graft model revealed the important role of the dermal scaffold to support the histoarchitecture of the skin, reach stability in long-term, and their capability to response to mechanical injuries with dynamic changes.

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HISTOLOGICAL CHARACTERIZATION OF A NOVEL ENGINEERED BIO-ARTIFICIAL ELASTIC CARTILAGE SUBSTITUTE

García-Martínez LA[1], Durand-Herrera D[1], Martín-Piedra MA[1], Fernández-Valadés R[2], Campos A[1], Sánchez-Quevedo MC[1], Garzón I[1], Carriel V[1]

[1] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[2] Division of Pediatric Surgery, University Hospital Complex of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA

Introduction: Elastic cartilage (EC) is a specialized connective tissue that provides structural support to the ear, epiglottis and larynx. EC could be affected by some pathological conditions such as cancer, congenital defects or traumatic injuries. The surgical repair of this tissue is difficult and costal hyaline cartilage is often used with variable success and several clinical complications. During the last years, synthetic biomaterials started to be used to generate customized substitutes to repair auricular defects. However, some of these biomaterials are poorly biocompatible and results were not optimal. Despite these advances it is still necessary to find biocompatible biomaterials that support the function of elastic cartilage-derived chondrocytes (ECDC). The aim of this study was to generate a novel engineered bio-artificial EC substitute by using human ECDC and nanostructured fibrin-agarose hydrogels (NFAH).

Material and methods: ECDC were isolated from healthy donors, expanded under standard culture conditions and cells from passage 2 were encapsulated into NFAH at 4,500 and 45,000 cells/ml (group A and B respectively). Constructs were kept under standard culture conditions and samples were harvested after 1, 2, 3, 4 and 5 weeks of ex vivo development for histological evaluation. Sections were stained with hematoxylin-eosin, alcian blue and orcein stainings, while collagens (type I and II) aggrecan, byglican and PCNA were identify by immunohistochemistry

Results: The histological and histochemical analyses revealed a progressive increase of the cells numbers in both groups. Alcian blue was negative, but orcein confirmed the synthesis of elastic fibers in group B. Cell proliferation index (PCNA) showed a decrease followed by an increase in both experimental groups. Immunohistochemistry demonstrated the synthesis of collagens (I and II), especially in the group B, while aggrecan and byglican were weakly produced in both groups.

Conclusions: This study demonstrated the suitability of NFAH in cartilage tissue engineering. This natural biomaterials supported cell proliferation and the synthesis of collagens, elastic fibers and proteoglycans. However, further studies are still need to obtain better functionally of these novel EC substitutes.

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IN VIVO BIOCOMPATIBILITY OF CROSSLINKED TISSUE-LIKE FIBRIN-AGAROSE BIOMATERIAL FOR TISSUE ENGINEERING APPLICATIONS. A SHORT-TERM STUDY

Carmona M[1], Campos F[1], Fernández-Valadés R[2], Alaminos M[1], Campos A[1], Sánchez-Montesinos I[3], Carmona R[4], Carriel V[1]

[1] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[2] Division of Pediatric Surgery, University Hospital Complex of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[3] Department of Human Anatomy and Embryology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[4] Department of Cell Biology, Faculty of Sciences, University of Granada, Spain

Introduction: Generation of biomaterials with adequate biomechanical and structural properties remains a challenge in tissue engineering. Earlier research has shown that chemical cross-linking techniques improved the biomechanical and structural properties of different biomaterials (1). Ex vivo studies have demonstrated that genipin enhance the biomechanical properties of fibrin agarose biomaterials, which have shown to be very useful in tissue engineering protocols. In this work we evaluated in vivo the biocompatibility of genipin-crosslinked biomaterials as a quality control for future clinical translation.

Material and methods: Acellular tissue-like fibrin-agarose biomaterials were generated from human plasma and type VII agarose. Then, chemical cross-linking was performed by using 0.25% and 0.1% genipin at 37ºC for 72h. Samples with an average size of 3x3x1.5 mm were implanted subcutaneously on the back of Wistar laboratory rats. Non-cross-linked biomaterials were used as controls. Results were analyzed histologically 12 days later using hematoxylin-eosin staining and Picrosirius.

Results: Histological analysis revealed high biocompatibility of the cross-linked scaffold, although an inflammatory infiltrate containing lymphocytes and neutrophils was detected at day 12. This infiltrate was higher in control non-cross-linked biomaterials and in the 0.1% genipin group. All biomaterials tended to degrade after 12 days, with higher degradation for 0.1% genipin biomaterials and the lowest levels of degradation for 0.25% genipin biomaterials.

Conclusions: Fibrin-agarose biomaterials cross-linked with 0.25% genipin could be a useful alternative to elaborate artificial tissues requiring a biomaterial that remains in vivo for at least 12 days with low levels of inflammation.

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ALOE VERA GEL AND HYBRID NANOFIBERS OF ALOE VERA ARE PERMISSIVE SUBSTRATES FOR NEURITE OUTGROWTH OF RAT DRG NEURONS IN VITRO

María M. Romero-Alemán[1], José E. Hernández-Rodríguez[1], José M. Pérez-Galván[1], Maximina Monzón-Mayor[1].

[1]Research group Neurogliociencia y reparación axonal (Nyra). Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas de Gran Canaria, Spain

Introduction: Aloe vera is a medicine plant that promotes wound healing. The nervous tissue is present in all the body organs and systems and regulates the conditions of health, the inflammation and the healing process. Nerve regrowth and proper reinervation of target tissues are important topics for the recovery of tissue homeostasis and restoration of functions. In addition, natural and synthetic materials mimetizing the microscopic structural organization of the extracelular matrix in healthy tissues can provide suitable bioscaffolds for cell growth and tissue regeneration. Our purpose is to study the neuritogenesis response of dorsal root ganglion (DRG) neurons in culture media containing different commercially available products of Canarian Aloe vera gel as well as electrospun hybrid nanofibers of Aloe vera.

Materials and Methods. Rat DRG neurons were cultured in DMEM/F12 (1:1) media (control group) and in the same media containing four commercial Aloe vera products at two dilutions (1/50 and 1/75) for 6 days. Commercial Aloe vera products were selected according to different composition of barbaloin (ranged between 2-180 ppm) and acemannan (ranged between 100-2000 mg/l). In addition, electrospun nanofibers containing only PHBV and a mixture of PHBV/Aloe vera were compared as scaffolds for neurite outgrowth in vitro. At the seveth day, the neuritogenesis response was detected using anti-neurofilament immunostaining. Data from the control group and the eight experimental groups were quantified using the image-J software (NIH, USA) and analysed statistically. Comparison of media and standard deviations was considered significant at $p < 0.05$.

Results: The neuritogenesis response was as high in control conditions as in presence of Aloe vera products containing high acemannan and/or low barbaloin composition, with independence of the dilution used. Moreover, neurite outgrowth was higher in presence of nanofibers of PHBV/Aloe vera than in presence of nanofibers of only PHBV.

Conclusions: We conclude that the concentration of acemannan and barbaloin are key factors involved in the permissiveness of the Aloe vera gel as substrate for the axonal outgrowth. Hybrid nanofibers containing Aloe vera are interesting resources for tissue engineering applications (patent ES P201600173; PCT ES 2017000029).

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IN VITRO CHONDROGENIC PROPERTIES OF ALGINATE-AGAROSE HIDROGELS

María Oliver [1], Lara Milián [1,2], José Javier Martín de Llano [1,2], María Sancho - Tello [1,2], Manuel Mata [1,2,3], Zaida Gregori [1], Javier Zurriaga [2,4], Carmen Carda [1,2,5].

[1] Departamento de Patología. Facultad de Medicina y Odontología. Universidad de Valencia, Spain.
[2] INCLIVA, Valencia, Spain.
[3] CIBERES, Instituto de Salud Carlos III, Spain.
[4] Hospital Clínico Universitario de Valencia, Spain.
[5] Ciber-BBN, Instituto de Salud Carlos III, Spain.

Introduction: Clinical management of large-size cartilage lesions is difficult due to the limited regenerative ability of the cartilage. Different biomaterials have been used to develop tissue engineering substitutes for cartilage repair including alginate. In this study, we explored the usefulness of the use of agarose in combination with alginate to generate a chondrocyte-friendly scaffold be used for in vivo articular regeneration.

Material and methods: Human articular chondrocytes were isolated and cultured in proliferation medium containing DMEM high glucose, 50 µg/mL ascorbic acid, 10% FBS, non-essential aminoacids, and antibiotics. After three passages, the cells were cultured with chondral differentiation medium containing DMEM, 1% ITS, 1% FBS and 10 ng/ml TGF beta for up to 4 weeks. Chondrocytes differentiation expression markers were studied by immunofluorescence using antibodies against type I and II collagens and aggrecan through a confocal microscope. Morphology changes were evaluated by fluorescence microscopy using rhodamine-phalloidin. Low-melting fusion point agarose (0.5-1%) was mixed with alginate (1.5-3%) and polymerized together. Human primary articular chondrocytes were cultured in the 3-D agarose-alginate scaffolds with chondral differentiation medium for 4-6 weeks. Chondrocytes differentiation expression markers as well as morphology changes were evaluated as above.

Results: After isolation, a rapid de-differentiation characterized by an acquisition of a fibroblast-like shape and a down-regulation of the expression of collagen II and aggrecan was observed. Re-differentiation of cultures was then induced using chondral differentiation cultured medium. Changes in cell morphology (the cells became rounded and started forming aggregates) as well as an increase in the expression of aggrecan first and type II collagen later were observed two weeks after changing the cell culture medium becoming significant after 3 weeks. Differentiation of chondrocytes was significant improved when the cells were cultured in 3D scaffolds. The cells cultured in alginate-agarose hydorgels secreted more type II collagen and aggrecan than those cultured in 2D systems. On the other hand hey form spherical organoids of 20-30 cells in which the release of COLII and aggrecan becomes maximal.

Conclusions: Agarose-alginate hidrogels are a friendly environment for chondral differentiation becoming a realistic scaffold for cartilage regeneration.

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ARTICULAR CARTILAGE REGENERATION INDUCED BY POLYCAPROLACTONE SCAFFOLD IN AN OVINE MODEL

María Sancho-Tello[1,2], Jürgen Solís-Ruiz[1], Amparo Ruiz-Saurí[1,2], Lara Milián[1,2], José J. Martín de Llano[1,2], Line Vikingsson[3], Santos Martínez Díaz[4], Joan C. Monllau[4], Gloria Gallego Ferrer[3,5], José Luis Gómez Ribelles[3,5], Carmen Carda[1,2,5].

[1]Department of Pathology, School of Medicine and Odontology, Universitat de València, Spain.
[2]Biomedical Research Institute (INCLIVA), Valencia, Spain.
[3]Centre for Biomaterials and Tissue Engineering (CBIT), Universitat Politècnica de València, Spain.
[4]Department of Orthopedic Surgery and Traumatology, Hospital del Mar, Autonomous University of Barcelona, Spain.
[5]Biomedical Research Networking Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain.

Introduction: The limited capacity of articular cartilage to repair itself is widely known and recognized as a handicap in the recovery of articular lesions. The purpose of this study is to approach cartilage regeneration under the effect of a synthetic implant in large mammals.

Material and Methods: Porous scaffolds made of biodegradable polycaprolactone (PCL) were synthetized attached to a poly(L-lactic acid) (PLLA) pin in order to improve cartilage regeneration. Six adult female Merino sheep underwent a surgery consisting on two 4-mm lesions at their femoral condyles with a central deeper hole in order to insert the implants. Animals were sacrificed 4,5 months after surgery, samples were obtained, decalcified for 4 months in Osteosoft®, and then cut in 2 halves through the lesions and embedded in paraffin. Five-µm sections were obtained and stained with hematoxylin-eosin, Masson's trichrome, picrosirius red for visual tissue references and alcian blue for cartilage identification. Microscopic characteristics of the neotissue on the lesion area were measured according to a modified O'Driscoll score, and a morphometric analysis was made using software (Image ProPlus 6.0) to assess the percentage of regenerated neocartilage tissue among other tissues (bone, undifferentiated). Data was exported and analyzed with IBM SPSS Statistics 20 applying a *t*-test after proving a normal distribution.

Results: All lesion areas presented an adequate repair process without implant rejection or fibrosis, showing an apparent effective integration with the surrounding native articular cartilage at the time studied. O'Driscoll score showed a high percentage areas of cartilage regeneration and integration with surrounding tissue, but no statistically significant difference were observed between the areas of regenerated cartilage and those of undifferentiated tissue (p value >0.05), while bone tissue represented less than 5% of total area.

Conclusions: Neotissue seems to differentiate into cartilage that matches the area occupied by undifferentiated tissue. This suggests that further research and longer postsurgical recovery times might show improved regenerative responses.

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3D-PELLETS OF CHONDROCYTE CULTURES WITH MICROSPHERES OF CHITOSAN AND/OR POLY(L-LACTIC) ACID FOR CARTILAGE REGENERATION

María Sancho-Tello[1,2], Lara Milián[1,2], Nadia Talón[1], Rosa M. Morales-Román[3], Manuel Mata[1,2,4], Mari Fe Mínguez[2,5], Gloria Gallego Ferrer[3,6], José L. Gómez Ribelles[3,6], Carmen Carda[1,2,6].

[1]Department of Pathology, School of Medicine and Odontology, Universitat de València, Spain.
[2]Biomedical Research Institute (INCLIVA), Valencia, Spain.
[3]Centre for Biomaterials and Tissue Engineering (CBIT), Universitat Politècnica de València, Spain.
[4]CIBERES, Spain.
[5]Department of Surgery, School of Medicine and Odontology, Universitat de València, Spain.
[6]Biomedical Research Networking Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain.

Introduction: Cartilage defects are difficult to repair due to the limited regeneration ability of this tissue. It has been shown that 3D-cultures improve the maintenance of hyaline cartilage chondrocyte phenotype better than 2D-cultures. Porous chitosan (CHT) and poly(L-lactic acid) (PLLA) scaffolds have proved useful in the cartilage regeneration when implanted in rabbits, but the difficulty in handling those rigid scaffolds has lead us to study the pellet culture of freshly isolated rabbit chondrocytes with scaffolds made of microspheres of CHT and/or PLLA.

Material and Methods: Chondrocytes alone or in combination with CHT, PLLA or both microspheres, were centrifuged to obtain cellular pellets, that were cultured either with proliferation or differentiation chondrocyte culture media for 4 weeks. Then, pellets were fixed with paraformaldehyde, cryopreserved, and 7-µm sections were obtained with the cryostat. The presence of a cartilage extracellular matrix was analyzed by using alcian blue staining to highlight the presence of glycosaminglycans (GAG), as well as by immunohistochemical detection of aggrecan.

Results: Image analysis showed a correlation between the histological detection of GAG and aggrecan immunostaining. Three-D cellular pellets cultured with proliferation media during 4 weeks showed less GAG and aggrecan than those cultured with differentiation media. When cells were cultured in the presence of one or both biomaterials (CHT and/or PLLA), cells grew around the microspheres, but it did not affect chondrocyte differentiation process since extracellular chondral markers had a similar presence than chondrocyte 3-D pellets cultured alone.

Conclusions: The presence of CHT and/or PLLA did not negatively affect the differentiation of chondrocytes when cultured in 3D-pellets. These scaffolds made of microsphere could facilitate the implant treatment of irregular cartilage lesions.

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IMMUNOHISTOCHEMICAL DETECTION OF FILAGGRIN AND HLA MARKERS ON A HETEROTYPIC MODEL OF BIOENGINEERED ARTIFICIAL SKIN

Martín-Piedra MA, Vela-Romera A, Zapater A, Mateu-Sanz M, Durand-Herrera D, Paes AB, Campos A, Garzón I

Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA

Introduction: Skin disruptions may alter the normal structure and functionality of the human skin, with the consequent loss of electrolytes and increased risk of microbial infections. Recently, tissue engineering has allowed the development of artificial human skin for clinical use. However, keratinocyte culture and expansion requires long culture periods. In order to solve this, in this study we aim to generate a bioengineered human skin model by using mesenchymal stem cells (MSC) as epidermal substitute.

Material and methods: Artificial skin stromas were developed using human dermal fibroblasts inside a fibrin-agarose scaffold. Human MSC from adipose tissue (ADSC), dental pulp (DPSC), Wharton's jelly (WJSC) and bone marrow (BMSC) were seeded on top of artificial stromas as an epithelial-like layer resulting in an artificial heterotypic human skin model. Constructs were grafted in athymic mice. After 4 weeks, animals were euthanatized for histological analysis (hematoxylin-eosin) and immunohistochemical detection of type I and II HLA molecules, and filaggrin as a mature epithelium differentiation marker.

Results: After 14 days, all groups showed a clear epithelial-like structure, mainly when ADSC or WJSC were used. At day 28, all mesenchymal stem cells showed a well development epithelial-like strata. DPSC and WJSC showed very low expression of HLA-I and HLA-II both in stromal and epithelial-like cells, suggesting that immune advantages of MSC remained after epithelial differentiation. Expression of these markers was evident at 7 days when ADSC and BMSC were used, but decreased as MSC differentiated. Furthermore, all MSC groups reached an epithelial phenotype as they expressed filaggrin after 28 days (even after 14 days in DPSC group).

Conclusions: MSC constitute an alternative cell source for epidermal regeneration as they could develop epithelial-like structures after 28 days, with epithelial phenotype and present very low expression of HLA molecules, which may lead to low risk of immunological rejection after allograft.

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GENERATION OF BIOENGINEERED SUBSTITUTES OF THE HUMAN ORAL MUCOSA CONTAINING MSC AS A NOVEL METHOD TO INCREASE IN VIVO BIOINTEGRATION

Mateu-Sanz M[1], Zapater A[1], Durand-Herrera D[1], Jaimes-Parra BD[1], España A[2], Alaminos M[1], Sánchez-Quevedo MC[1], Garzón I[1]

[1] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[2] Cleft lip and palate Treatment Unit, University Hospital Complex of Granada, Spain

Introduction: Over the last few years, researchers have attempted to develop an oral mucosa substitute able to reconstruct oral mucosa defects. However, vascularization of tissue-engineered oral mucosa (TEOM) models is still a challenge. Vascularization has been addressed by the use of endothelial a non-endothelial cells. In this study, we have used mesenchymal stem cells (BMSCs) to determine their in vivo differentiation potential to endothelial cells.

Material and methods: Two different models of TEOM were generated in the laboratory using fibrin-agarose biomaterials. The first model contained 70,000 human oral mucosa fibroblasts immersed in the biomaterial. The second model had 35,000 human oral mucosa fibroblasts and 35,000 BMSCs. Human oral mucosa keratinocytes were cultured on top of both artificial stromas and submitted to air-liquid culture technique for 2 weeks to promote epithelial stratification. Both TEOM were grafted in vivo on Foxn1nu athymic mice. All animals were euthanized after 15 days and histological analysis (hematoxylin-eosin) was performed. Immunohistochemical detection using a specific anti-human mitochondria antibody was used to identify human cells.

Results: Our results showed a large amount of blood vessels in both study groups, suggesting that both types of TEOM were able to form capillary-like structures. However, the amount of capillary-like structures was higher in TEOM using BMSCs cells in comparison with TEOM with stromal fibroblasts only.

Conclusions: The use of stromal BMSCs improved neovascularizacion and the regeneration process of TEOM substitutes at in vivo level. These results suggest that the use of BMSCs could accelerate blood supply to the grafted tissues after transplantation and could therefore increase the success rates of the implant.

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FUNCTIONALIZATION OF FIBRIN-AGAROSE ACELLULAR SCAFFOLDS BY THE INTRODUCTION OF HUMAN ORAL MUCOSA TISSUE EXPLANTS

Mateu-Sanz M, Zapater A, García JM, Quintero-Campos P, Vela-Romera A, Sánchez-Quevedo MC, Garzón I, Martín-Piedra MA

Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA

Introduction: Improvement of scaffolds used in tissue engineering will contribute to a better development of bioartificial human tissues, and functionalization arises as an efficient way to enhance scaffold properties. Functionalization can be achieved by the introduction of functional molecules such as extracellular matrix (ECM) components, drugs, peptide sequences or/and growth factors that can interact with cells when they are released. In this work, we have functionalized and evaluated human oral mucosa constructs by the introduction of human tissue explants containing relevant ECM molecules.

Material and methods: Fibrin-agarose acellular scaffolds were generated by tissue engineering and functionalized by the introduction of small human oral mucosa tissue explants during the fabrication process. Scaffolds were kept in culture for 4 weeks. Collagen fibers were identified by picrosirius staining and type I-collagen immunohistochemistry, and non-fibrillar ECM components (glycoproteins and proteoglycans) were analyzed by PAS and alcian blue staining. Vimentin immunohistochemistry was used to identify stromal cells. In all cases, results were quantified from 0 -no expression- to 100% -maximum expression- by analyzing staining signal intensity using ImageJ software using human native oral mucosa samples as controls.

Results: Vimentin-positive cells tended to remain into the explants during the first 2 weeks, but migrated into the hydrogel at weeks 3 and 4 of ex vivo development. Levels of expression of non fibrillar ECM components (proteoglycans and glycoproteins) were similar in the bioartificial tissues and in control native oral mucosa stromas after 3 and 4 weeks. For fibrillar proteins, collagen showed low expression (26.69% in week 3 and 36.22% in week 4) as compared to controls (67.01%).

Conclusions: Human oral mucosa tissue explants may be used as a functionalization technique to generate bioengineered stromas containing key ECM components that are present in native tissues. Stromal fibroblasts are able to migrate into the biomaterial and could contribute to tissue regeneration.

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THE IMPORTANT ROLE OF CELL DIFFERENTIATION IN THE REPAIR OF CRITICAL MANDIBULAR DEFECTS WITH IMPLANTS OF CONSTRUCTS NANOHYDROXYAPATITE/AGAROSE

Natalio García-Honduvilla [1,2,3], Alejandro Coca [1], Miguel A Ortega [1,3], Sergio Ramírez-Varela [1], Juan Peña [3,4], María V Cabañas [3,4], Jesús Román [3,4], María Vallet-Regí[3,4], Julia Buján [1,3].

[1]Departments of Medicine and Medical Specialities, University of Alcalá,Alcalá de Henares, Spain [2]University Center of Defense of Madrid (CUD-ACD),Spain, [3]Networking Biomedical Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN),Madrid, Spain, [4]Department of Inorganic Chemistry and Bioinorganic, Complutum University, Madrid, Spain.

Introduction: Reconstruction of bone by autologous bone transplant has been established as the “gold standard” due to the osteogenic properties of the transplanted material. However, bone grafting is only applicable to relatively small defects. The objective of this work is to evaluate the efficiency of a biomaterial (nanocrystalline carbonated hydroxyapatite reinforced agarose hydrogel) as a scaffold for bone regeneration in critical mandibular defects.

Material and Methods: The GELPOR3D shaping method can be used to create what are described as ceramic reinforced hydrogels where the structural components maintain both their original microstructural and textural properties and thus their functionality. Using this method, nanohydroxyapatite/agarose (80/20%) scaffolds containing undifferentiated mesenchymal cells of adipose tissue (MSCAT) or osteogenic differentiated cells (O-MSCAT) were prepared. Histological (von Kossa, alkaline phosphatase) and immunohistochemical techniques for RUNX2, OCT3/4, Nanog, and osteopontin were used for characterize the cells. For in vivo studies, we created (n=6) four critical mandibular defects (35 mm x 15 mm) (2 hemiarcade) on which the scaffold plus cells was implemented (total number of defects = 24). The animals were sacrificed after 6 months. The samples obtained were decalcified and processed for extracellular matrix examination using micro-CT techniques, PCR and immunological staining (osteopontin, osteocalcin, RUNX-2 and Collagen I and III).

Results: MicroCT analysis showed an increase of ~20% in the size of the defect without scaffolds. In contrast, when scaffolds and MSCAT or OMSCATS constructs were implanted, we obtained a more than 50 % diminution in defect size. Furthermore, evaluation of ECM area showed a 40 % increase in bone regenerated area.

Conclusions: nanohydroxyapatite/agarose scaffolds containing undifferentiated mesenchymal cells offer a promising approach for repairing critical mandibular defects.

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GENIPIN ENHANCES THE MECHANICAL PROPERTIES OF DECELLULARIZED NERVES FOR TISSUE ENGINEERING

García-García O, Campos F, Chato J, Paes AB, Quintero-Campos P, Sánchez-Quevedo MC, Carriel V, Campos A

Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA

Introduction: Decellularization techniques have been widely used as strategy for organ reconstruction, including peripheral nerve. As the natural extracellular matrix provides an ideal environment for topographical, electrical and chemical cues to the adhesion and proliferation of neural cells, decellularized nerve allografts could be used for the generation of peripheral nerve substitutes. However, the decellularization process may affect the structure, composition and mechanical properties of decellularized nerves, implying the need of implementing subsequent methods able to enhance the properties of these models. The current study aimed to investigate the effects of genipin as a chemical cross-linker agent on the mechanical properties of decellularized nerves.

Material and methods: Wistar rat sciatic nerves were decellularized by a protocol that combines the use of a detergent (1% Triton X-100) with several enzymes (DNase, RNase and Trypsin). Then, decellularized nerved were washed in PBS and subjected to chemical cross-linking using two concentrations (0.1 and 0.25%) of genipin. Non-cross-linked decellularized nerves were used as controls. Biomechanical properties of each sample were measured by tensile test using an Instron biomaterial analyzer.

Results: The use of genipin as cross-linker agent at concentrations of 0.1% and 0.25% showed a significant ($p=0.046$) increase of the Young modulus as compared to control decellularized nerves (9.3±2.7MPa for controls and 16.9±1.6MPa for 0.1% genipin and 17.0±3.8MPa for 0.25% genipin). No differences were found between both genipin concentrations. The same phenomenon were found for the charge and stress at fracture.

Conclusions: Treatment with genipin improves the mechanical properties of decellularized sciatic nerves. Therefore, cross-linked decellularized nerves could be used in nerve regeneration. However, further in vivo preclinical studies are still required.

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BIOMECHANICAL CHARACTERIZATION OF TISSUE-LIKE CONSTRUCTS BASED ON AGAROSE HYDROGELS

Quintero-Campos P[1], Campos F[1], Santisteban-Espejo A[2], Campos A[1], Carriel V[1], Alaminos M[1], Sánchez-Quevedo MC[1], Fernández-Valadés R[3]

[1] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[2] Servicio de Hematología y Hemoterapia, Hospital Universitario Puerta del Mar de Cádiz
[3] Division of Pediatric Surgery, University Hospital Complex of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA

Introduction: The use of agarose has been recently introduced in skin, cornea, nerve and oral mucosa (1) tissue engineering protocols to improve the biomechanical properties of natural hydrogels, especially fibrin. This polysaccharide is extracted from marine algae and has the ability to form thermally reversible gels. Several types of agarose are commercially available, with type I and type VII being the most commonly used agaroses in medical applications. However, the biomechanical properties of each agarose type used in tissue engineering have not been elucidated to the date. The aim of the present study is to determine the biomechanical behavior of tissue-like models elaborated with both types of agarose.

Material and methods: First, tissue-like constructs were generated using type I and type VII agarose hydrogels at a final concentration of 2%. Then, the biomechanical properties of these constructs were evaluated using an Instron material testing device after 2 and 16 days in culture as previously described (2). In each case, Young modulus, stress at break and strain at break were obtained by selecting the point of the strain-strain curve where the gel fracture occurred.

Results: Type I agarose hydrogels were characterized by a smaller Young modulus (0.0055±0.0009MPa at day 2 and 0.0658±0.0112MPa at day 16) as compared to type VII agarose (0.0265 ± 0.0680MPa at day 2 and 0.1071±0.0245MPa at day 16), suggesting that type I hydrogels tolerated less compressive strength than type VII agarose hydrogels. In addition, the gels tended to lose elasticity over time, with an increase in Young modulus and, therefore, resisted lower compressive forces. Differences were statistically significant for the comparison between agarose types and time of development in culture.

Conclusions: Our results support the use of type VII agarose for the elaboration of tissue-like constructs requiring high elastic properties such as the human cornea, nerve, skin and oral mucosa.

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CHARACTERIZATION OF THE ADHESION AND GROWTH OF HUMAN DENTAL PULP STEM CELLS AND MACROPHAGES ON ELECTROSPUN WEBS

Rubén Salvador-Clavell[1], Bruno Marco[2], Carlos Pascual[2], Manuel Mata[1,3], María Sancho-Tello[1], Lara Milián[1], Carmen Carda[1,4], María Blanes[2], José Javier Martín de Llano[1].

[1]Department of Pathology and Health Research Institute of the Hospital Clínico (Incliva), Faculty of Medicine and Dentistry, University of Valencia, Valencia, Spain.
[2]Research Group of Technical Finishing, Biomedicine and Health of the Technical Research Center AITEX, Alcoy, Spain.
[3]Ciberes, Instituto de Salud Carlos III, Spain.
[4]Ciber-BBN, Instituto de Salud Carlos III, Spain.

Introduction: Oral cavity diseases could benefit from tissue engineering processes producing cellularized scaffolds favoring bone tissue remodeling. Herein we describe preliminary results obtained by using electrospinning produced webs as scaffolds for the growth of both human dental pulp stem cells (hDPSC), which can differentiate into osteoblasts, as well as human monocytes, which can differentiate into macrophages and osteoclast-like cells.

Material and Methods: A1 webs were produced by electrospinning with a 50:50 mix of DL-lactide:glycolide. A2 webs were produced adding hydroxyapatite nanoparticles (nHAp) to the DL-lactide:glycolide mix. hDPSC and THP-1, a monocytic cell line, were cultured following standard procedures. Phorbol 12-myristate-13-acetate was added to THP-1 cell culture media to induce macrophage differentiation. Webs sections were incubated with hDPSC and THP-1 cell culture media at 37 ºC for 24 h, 1 and 3 weeks under continuous shaking and MTS viability assays were carried out using aliquots of those conditioned media. Cell adhesion and proliferation were verified by culturing cells on webs sections clamped on the bottom of 8-well Millicell EZslide cell culture slides and fluorescence microscopy observation of the samples after eosin and DAPI staining.

Results: nHAp particles were consistently incorporated to the electrospun nanofibers. SEM analysis showed that A2 nanofibers had a smaller diameter than A1 nanofibers. Twenty four-h and 1-week conditioned media from both A1 and A2 webs did not affect the viability of hDPSC and macrophage cells. hDPSC cultured in 3-weeks conditioned media from both A1 and A2 webs showed a lower viability. Macrophages adhered to both A1 and A2 webs. hDPSC adhered to the webs and proliferated, displaying an elongated morphology following the nanofibers shape.

Conclusions: nHAp particles can be incorporated to electrospun nanofibers without greatly altering their morphological properties.
A1 and A2 webs did not release substances toxic to THP-1 cells. hDPSC could be slightly more sensitive to substances released at the most stringent condition tested.
A1 and A2 webs are reliable scaffolds for the adhesion of macrophages and the adhesion and proliferation of hDPSC.

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A PRELIMINARY STUDY OF THE EFFECT OF MAGNETIC FIELDS ON 3D-CULTURED HUMAN ARTICULAR CARTILAGE CHONDROCYTES

Rubén Salvador-Clavell[1], José Javier Martín de Llano[1], Manuel Mata[1], Lara Milián[1], María Oliver[1], Irene López[2], Jose-Manuel Rodríguez-Fortún[2], Javier Orús[2], Mohamed Hamdy Doweidar[3,4], María Sancho-Tello[1], Carmen Carda[1,4].

[1]Department of Pathology and Health Research Institute of the Hospital Clínico (Incliva), Faculty of Medicine and Dentistry, University of Valencia, Valencia, Spain.
[2]Instituto Tecnológico de Aragón (ITAINNOVA), Zaragoza, Spain.
[3]Mechanical Engineering Department and Aragon Institute of Engineering Research (I3A), University of Zaragoza, Spain.
[4]Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Instituto de Salud Carlos III, Spain.

Introduction: Mechanical stimulation can improve articular cartilage regeneration. Extracellular magnetic particles moving under the effect of an external magnetic field can reproduce the mechanical forces by acting on the chondrocytes. We have designed and fabricated a device conceived for generating a controlled magnetic field with homogeneous characteristics, which interacts with superparamagnetic beads present in the extracellular matrix. Herein we present preliminary results obtained applying a magnetic field on cultured human chondrocytes.

Material and methods: The device was designed for maximizing the magnetic flux gradient, while its magnitude always ensured the magnetization of the beads. Human articular cartilage chondrocytes were incorporated to 3 % alginate scaffolds and cultured in proliferation cell culture media into 0.5-mL microtubes. A magnetic field (30 T/m, pulse direction changing every 3 s) was applied as a daily schedule of 4 treatment sequences (1 h irradiation/1 h resting each). Control samples were maintained always inside the cell incubator whereas non-irradiated control samples were kept in the device during treatment. After 1-week, viability assay (MTS) was carried out, and cell nuclei morphology was studied by fluorescence microscopy after DAPI staining.

Results: The device generated a gradient of 30 T/m in the working area (15x15 mm^2) and was able to produce 14 nN on magnetic particles of 20 μm diameter. To work with different samples, a displacement actuator was implemented. Cells incorporated to alginate scaffolds had a spherical morphology. After 1-week irradiation, cells showed a slightly higher viability than those kept inside the incubator. Furthermore, irradiation did not affect cell viability, and no differences were found in cell nuclei morphology and integrity.

Conclusions: The fabricated device can be effectively used to irradiate cells since these incorporated to an alginate scaffold can be irradiated for at least 1-week without affecting cell viability or morphology. The addition of paramagnetic particles to cultured cells would allow that simulating mechanical forces could act on cells *in vivo*.

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HANGING DROP AND *PELLET CULTURE* MODELS FOR CHONDROGENIC DIFFERENTIATION OF ADIPOSE-DERIVED STEM CELLS

Verónica Zubillaga Marañón[1], Ana Alonso-Varona[1], Patricia Garrido-Pascual[1], María Burón Aizpiri[1], Teodoro Palomares Casado[2].

[1]Department of Cell Biology and Histology, Faculty of Medicine UPV/EHU, Leioa, Spain.
[2]Department of Surgery, Radiology and Physic Medicine, Faculty of Medicine UPV/EHU, Leioa,Spain.

Introduction: Cartilage is an ideal candidate for tissue engineering (TE) due to its limited ability of self-repair. Human adipose-derived stem cells (hASCs) have been widely used in cartilage TE because of their differentiation capacity toward chondrogenic phenotype. In this situation, hASCs tend to form a three-dimensional (3D) environment, which attempts the direct cell-cell interactions observed in the cartilaginous condensations during the embryonic development. Currently, two main strategies are in use to form 3D spheroids: *hanging-drop (HD)* and *pellet culture (PC)* techniques. The aim of this study was to compare and evaluate the chondrogenic differentiation level of hASCs 3D spheroids formed by both *HD* and *PC* techniques in order to adapt the biological component to the suitable porous scaffold.

Materials and methods: *Hanging-drop:* hASCs at a density of $15x10^3$ cells/30 µl in complete culture media were seeded in petri dishes. After 48 h, fresh complete culture media or chondrogenic differentiation media were added.
Pellet culture: hASCs were placed in polypropylene tubes at a density of $2,5x10^4$ cells in 300 µl of complete culture media or chondrogenic differentiation media and centrifuged at 400 g.
To evaluate chondrogenic differentiation (extracellular matrix deposition), histological analyses (hematoxilin/eosin, Masson´s trichrome, safranin-O and alcian blue) were performed at 7, 14 and 21 days.

Results: As expected, *PC* spheroids present a larger size compared to that obtained in *HD* spheroids. The histological evaluation of both, *HD* and *PC* spheroids, showed a good differentiation level with a progressive increase of collagen and glycosaminoglycans (GAGs) deposition over time. However, *HD* method shows a higher presence of both collagen and GAGs, as can be seen through positivity in Masson´s trichrome and safranin-O stains, respectively.

Conclusions: Both hASCs 3D culture models provide an excellent pro-chondrogenic microenvironment. However, as *HD* formed spheroids have smaller size and present a higher level of cartilage specific markers, this method would be more suitable to create tissue constructs when combined with an adequate biomaterial.

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NANOSTRUCTURED FIBRIN-AGAROSE BIO-ARTIFICIAL NERVE SUBSTITUTES SUPPORT PERIPHERAL NERVE REGENERATION IN RATS

Carriel V[1], Miralles E[2], Sáez JA[2], Paes AB[1], Katati MJ[3], García JM[1], Alaminos M[1], Campos A[1]

[1] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[2] Division of Clinical Neurophysiology, University Hospital Complex of Granada, Spain
[3] Division of Neurosurgery, University Hospital Complex of Granada, Spain

Introduction: Peripheral nerves are often affected by traumatic injuries and critical defects represent a serious problem due to their high incidence, poor functional recovery and the lack of effective treatments. Tissue engineering emerged as a promising alternative for the generation of functional substitutes to repair damaged organs. Our group demonstrated that the use of fibrin-agarose hydrogels (FAH) with mesenchymal stem cells as intraluminal fillers of conduits improved peripheral nerve regeneration in rats. The aim of this study is to design and evaluate in vivo a bio-artificial nerve substitute for nerve repair by using nanostructured FAH and autologous adipose-derived mesenchymal stem cells (ADMSCs).

Material and methods: ADMSCs were isolated from 15 male Wistar rats and used to generate autologous nanostructured FAH bio-artificial nerve substitutes (NFABNS). After that 10-mm of the left sciatic nerve was removed from each rat and repaired by using NFABNS, NFABNS as intraluminal fillers of collagen conduits (NFABNS-C) and autograft as control. Animals were subjected to electromyography and clinical tests at 6 weeks and 12 weeks. After 12 weeks, implants were removed and the peripheral nerve regeneration was determined with MCOLL histochemical method and immunohistochemistry for S-100 and GAP-43.

Results: Electromyography and clinical test showed a progressive reinnervation and functional recovery from 6 to 12 weeks in all groups, especially autograft. The use of NFABNS showed better clinical and functional results as compared to NFABNS-C group. Histology confirmed that the peripheral nerve regeneration cross the gap in all groups. In FABNS group the regeneration was associated to the surface of the implant and also between the surrounded muscle fibers, while in FABNS-C the regeneration was restricted to the lumen and less abundant.

Conclusions: This in vivo study demonstrates that nanostructured FAH could be used to generate biologically active bio-artificial substitutes for peripheral nerve repair. These substitutes were able to bridge a critical nerve gap and support peripheral nerve regeneration and functional recovery.

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DEVELOPMENT OF NOVEL MAGNETIC-RESPONSIVE TISSUE SUBSTITUTES. AN IN VIVO BIOCOMPATIBILITY EVALUATION

Carriel V[1], Campos F[1], Bonhome AB[2], López-López MT[2], Alaminos M[1], Campos A[1], Fernández-Valadés R[3], Rodríguez IA[1,4]

[1] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[2] Department of Applied Physics, University of Granada, Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[3] Division of Pediatric Surgery, University Hospital Complex of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[4] Cátedra B de Histología, Facultad de Odontología, National University of Cordoba, Argentine

Introduction: Magnetic nanoparticles (MNPs) are used in tissue engineering scaffolds to support the regeneration of damaged native human tissues (1,2). The objective of this in vivo study is to evaluate the biodistribution and biocompatibility of magnetic tissue-like constructs based on fibrin/agarose hydrogels (FAH).

Material and methods: We fabricated magnetic tissue-like constructs based on FAH containing MNPs, which were subcutaneously implanted in the connective tissue of 5 adult male Wistar rats. As controls, FAH without MNPs was implanted in other 5 animals. After 7 and 21 days, animals were subjected to magnetic resonance imaging (MIR) and histological analyses. For histology, the implant area, liver, kidneys, spleen and lymph nodes were stained with routine methods and Perl's histochemical method (for iron).

Results: MIR showed hyper-intense areas in the region of FAH-MNPs implantation at 7 and 21 days, but signs of the presence of MNPs were not observed in distal organs. In all cases, organs were of normal appearance, without any appreciable damage or morphological changes. Histology showed the presence of Perl´s positive areas in the FAH-MNPs group and confirmed a high amount of MNPs in the implantation region, but not in the distal organs. Only a local macrophage and lymphoplasmocytic-rich inflammatory reaction was observed at day 7 around the implants in FAH and FAH-MNPs groups, but this inflammatory reaction decreased at day 21. Indeed, all organs were histologically normal during the 21 days.

Conclusions: Based on MRI and histological analyses, no measurable migration of particles took place after 21 days of implantation. For these reasons, we believe that magnetic tissue-like FAH-MNPs constructs could be useful in local treatments, as slow drug delivery system and other future tissue engineering applications. Analysis at longer time periods is still in need to elucidate the potential clinical application of this strategy.

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OUTLINE OF CONTENTS IN CELL BIOLOGY BY THE ONLINE WORKING OF GOOGLE PRESENTATIONS

Ana Coto-Montes, Ignacio Vega-Naredo, Juan C. Bermejo-Millo, Zulema Pérez-Martínez, Beatriz Caballero

Department of Morphology and Cell Biology, Faculty of Medicine, University of Oviedo

Introduction: The present project proposes the realization of didactic schemes by the students of the main thematic contents included in the subject of Cell Biology, belonging to the subject "Cell Biology and Histology" (Degree of Biology), through the online sharing of presentations of Google.

Material and methods: For the project, the students only needed a computer with internet connection and a Gmail personal account. The bibliographic resources available were those included in the theoretical classes of the subject, which were accessible through the virtual campus of the University, as well as those references recommended by the teaching guide of the subject, the latter available in the library of the Faculty of Biology. The students were informed about the project and they were asked for the consent for their participation. Then, the participants were divided into working groups of about 3-4 students and topics were assigned for each group. Finally, Google presentations were created and invitations were sent by e-mail to each student participating in the project.

Results: The project was carried up by 3 main working phases: start, execution and final evaluation. The most important activities performed by the students corresponded to the execution phase. The project had a percentage of participation of 64% (49 students of a total of 77). However, the percentage of success was of 51%, since only a total of 25 students completed their online Google presentation. Among the main inconvenient detected, we should note a low working collaboration among the students belonging to the same group as well as a deficient implication in online tutoring. Notably, most of the students did a longer presentation of the topic instead of a didactic scheme. In any case, all presentation completed were positively valued.

Conclusions: Strategies for innovation in teaching based on the e-learning methodology could significantly increase learning competences of university students. However, to guarantee a valuable collaborative learning, new strategies should be considered to motivate the students and have a higher control of the online working.

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INCORPORATION OF THE SMARTPHONE AND FACEBOOK AS COLLABORATIVE LEARNING TOOLS INTO HISTOLOGY LABORATORY TEACHING

Ana Coto-Montes, Yaiza Potes, Adrian Rubio-González, Beatriz Caballero, Ignacio Vega-Naredo.

Department of Morphology and Cell Biology, University of Oviedo, Oviedo, Spain.

Introduction: The interpretation of histological specimens in laboratory sessions is a complicated task since it requires knowledge of microscopy methodologies. The understanding of three-dimensional structures from two-dimensional images is achieved only after observation of many histological preparations. However, during the practical sessions there is not enough time for the interpretation of a large number of different slides. Therefore, it is necessary to implement new strategies that allow students to visualize a greater number of histological images. In recent years, the use of smartphones with photographic devices and social networks has become widespread. Based on these considerations, we designed a new learning situation for laboratory sessions.

Material and methods: During the observation of histological sections, we have allowed the students of Histology (Biology degree) from year 2016-17 to capture their own images using their mobile devices to share them in a closed group on Facebook, enabling the dissemination of these images among his colleagues and the establishment of discussions that would help to socialize knowledge. Our role as teachers was to guide students to identify structures, recognize the best fields to be photographed and guide the discussion and selection of images on Facebook. To evaluate the effectiveness of this approach, academic performance was compared among participating and non-participating students.

Results: Participating students (n=67) showed a greater academic performance than non-participating students (n=37) ($p<0.001$). In addition, the percentage of students who were awarded with an academic qualification higher than 7/10 was 51% in the non-participating group while in participating students was 85%. When comparing with (non-participating) students from preceding years (2014-15 and 2015-16), the academic qualifications were also significantly higher ($p<0.001$).

Conclusions: The use of collaborative learning tools including technologies such as photographic mobile devices and social networks in histology laboratory teaching carries a greater academic performance.

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RELATIONSHIP BETWEEN LEARNING STYLE PREFERENCES AND ACADEMIC SUCCESS OF MEDICAL UNDERGRADUATE STUDENTS IN THE CELL BIOLOGY AND HISTOLOGY PRACTICES.

Eva del Valle-Suárez[1], Eva Martinez-Pinilla[1], Jorge Tolivia-Fernández[1], Ana Navarro-Incio[1]

[1]Department de Morphology and Cell Biology, Faculty of Medicine and Health Sciences, University of Oviedo, Spain

Introduction: Learning style is an individual's characteristic way to perceiving, processing and retaining new information that vary from pe rson to person du e to differen ces in co gnitive processing. During last years, edu cational research efforts hav e been fo cused on improve teaching techniques in order to fa cilitate student learning. T his fact takes on particular importance in Cell Biology and Histology (CBH) su bject of Medi cal Degree at the Unive rsity of Oviedo in which the observation and analytical drawing of microscopy slide s is one of the m ain objectives to pass the co urse. The ai m of this work was to study the pote ntial influence of students' different learning style preferences in their academic success in the CBH practices.

Material and Methods: A cross sectional study was conducted on the students of the first ye ar of Medical Degre e at the University of Oviedo. The VARK questionnaire was used to cla ssify the learning preferences as Visual (V), Aural (A), Reader/Writer (R), Kinesthetic (K) and Multimodal (M) for th ose students with more than one m odal preference. The questionnaire completion was voluntary. The VARK que stionnaire results were contrasted with the pra ctical exam score.

Results: Our data sh owed that majorit y (77%) of t he Medical students had unimodal VARK preferences. Among them, the m ost common preference was Aural type (37%), followed by Kinesthetic (26%), Read er (11%) and Visual (2%). The re st of the students (23%) had more than one mo dal preference. The findings also reve aled mean differences in the academ ic achievement between unimodal students. Curiously, visual learners sco red the highest mean followed by kinesthetic, aural and reader ones. Interestingly, the mean score of the multimodal students was very close to the lowest unimodal preference score indi cating that learni ng preferences are equally important in study accomplishment.

Conclusion: Learning styles and teaching strategies influence students' academic achievement in the practical part of the CBH subject of the Medical Degree.

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LEARNING PREFERENCES OF HEALTH SCIENCES STUDENTS AT THE UNIVERSITY OF OVIEDO

Ana Navarro-Incio[1], Eva del Valle-Suárez[1], Eva Martinez-Pinilla[1], Jorge Tolivia-Fernández[1]

[1]Department de Morphology and Cell Biology, Faculty of Medicine and Health Sciences, University of Oviedo, Spain

Introduction: Cell Biology and Histology (CBH) is a subject taught in all Health Sciences Degrees at the University of Oviedo. One of the main tools in the teaching-learning process of CBH is the observation and analytical drawing of microscopy slides. In recent years, we have observed a decrease on the marks obtained by the students in the practical part of the subject. In order to improve the se results, we decided to study the learning preferences of Medical, Nursing and Physiotherapy students.

Material and Methods: A VARK (Visual, Aural, Reader/Writer, Kinesthetic) questionnaire was provided to the students of the Degrees referred above, being the completion voluntary. With the obtained results the students were classified according to the predominant VARK category, adding a fifth one (Multimodal, M) for those students with more than one modal preference. The scores were also contrasted with sex and type of previous education.

Results: The participation rate was 62%. A first analysis of the data showed that 80% of students had a unimodal preference while 19% were multimodal. Medical students were mainly Aural type, followed by Kinesthetic and Multimodal. In Physiotherapy and Nursing Degrees the most frequent preference was Kinesthetic, followed by the Aural and Reader. Additional data analysis have shown that learning preference seems to be influenced by sex and it also appears that learning styles are closely related to the Degree chosen by the students.

Conclusion: Learning preferences seem to be related to the career choice of the Health Sciences students, since the most frequent learning model in the Degrees of Physiotherapy and Nursing is Kinesthetic whereas it is Auditory for Medicine, regardless of sex or type of previous education.

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Medical students respond positively to the implementation of AR technology in their studies in Human Histology

AM. Moreno-Fernández[1], L. Macías-García[1], J. Benoit-Perejón, A. Fernández-Rodriguez[1], M. de Miguel-Rodríguez[1].

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

Introduction: Augmented reality (AR) is formed as a technology with great potential for academic teaching at university, offering the possibility of combining digital information with real time physics across different technological devices (*tablets*, *smartphones*, glasses…). Our goal in this study was to discover the degree of motivation that the use of AR technology could arise in medical students and study how to implement it efficiently.

Material and Methods: Analyisis of the degree of motivation in students was carried out by passing a survey developed by Keller (2010), the "*Instructional Material Motivational* Survey" (IMMS). The study sample was comprised by first year students of the Degree of Medicine in the University of Seville (Spain), who were studying "Human Histology". The evaluated AR objects (Stomacharus, Lungarus, Liverarus, Kidneyarus, Heartarus) were generated by the audiovisual department of the University of Seville (SAV) and the professors in the Department of Normal and Pathological Citology and Histology that present this communication.

Results: We can emphasize that this experience caught the attention of students and that the AR objetcts arose their curiosity, helping them maintain their attention and enjoy the overall experience while simultaneously presenting them content relevant to their interests. These apps allowed us to increase the amount of information provided to the students in their notes for Human Histology in an integrated way and with clinical applications, allowing them to interact with different organs in 3D while they enjoyed viewing educational videos with macroscopic and microscopic levels of detail.

Conclusions: This study helps to show the motivation, attention, confidence, relevancy and satisfaction that arise thanks to the interaction with AR objects when these are applied in the academic field. Moreover, AR presents itself as a great tool for teaching in medical students and it is easy to implement in their studies.

EVALUATION OF ENGLISH AND FRENCH HISTOLOGICAL TERMS IN MEDICAL STUDENTS

Ruyffelaert A[1], Campos F[2], Martín-Piedra MA[2], Rodríguez IA[2,3], García JM[2], Crespo PV[2], Campos A[2], Carriel V[2]

[3] Department of Linguistics, Ghent University, Belgium and Department of Didactics of Language and Literature, University of Granada, Spain
[2] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[3] Cátedra B de Histología, Facultad de Odontología, National University of Cordoba, Argentine

Introduction: Globalization encourages us to acquire linguistic competences for our professional performance or to get access to scientific or technical information. It is especially important in medicine and academic careers. Unfortunately, the acquisition of specific terminology in foreign languages is difficult and generally forgotten in languages courses. The aim of this study is to explore a novel teaching strategy to promote a passive acquisition of histological terms in English and French in medical students. In addition, we evaluated the gender-related differences, student perception and the impact of this activity on the student's academic performance.

Material and methods: This activity was carried out with volunteer medical students enrolled in the course of Histology at the University of Granada (Spain). Students were subjected to multiple choice histological tests in English, French and Spanish (as control), followed by a perception survey.

Results: This activity was performed by a high number of volunteers (73% in French, 84% English and 100% Spanish) which showed significantly higher scores in English as compared to French. 71% of students found the test useful for English and 52% for French. In addition, students who did these tests obtained higher scores in the course of histology as compared to the students that did not participate.

Conclusions: This study showed that students have higher knowledge of scientific terms in English as compared to French. In addition, most of these students agreed to continue doing innovative didactic strategies to improve their scientific languages skills. However, long-term studies are still needed in order to demonstrate a real impact of this activity in the acquisition of scientific terms in foreign languages as wells as in their histological knowledge.

THE USE OF SURVEYS AS AN ASSESMENT OF TEACHING ACTIVITY IN THE TRAINING OF HUMAN HISTOLOGY

Blanca Ibarra[1], Miguel A. Ortega[1], Gemma Pascual[1], Angel Asúnsolo[2], Lara Cristóbal[1], Andrés A. Maldonado[1], Julia Buján[1], Natalio García-Honduvilla[1,3].

[1]Departments of Medicine and Medical Specialities, Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares. Networking Biomedical Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain. [2]Department of Surgery, Medical and Social Sciences, Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares, Madrid, Spain. [3]University Center of Defense of Madrid (CUD-ACD), Spain.

Introduction: The incorporation of new technologies in teaching innovation has allowed us to develop tools for the improvement of this field at a very low cost and with a great ease for the student-teacher interaction. In this project, we have considered the improvement of teaching by assessing the structure of different thematic blocks of two subjects: Biology: Medical Cytology and Cytogenetics and Human Histology.

Material and methods: The distribution of these subjects in the first and second semesters of the same academic year has allowed us to follow a full promotion throughout the year, through the creation of telematic surveys that evaluated parameters such as the distribution of hours, the complexity of the thematic block or their previous knowledge of the subject, among others. The surveys were completely anonymous and incorporated a pseudonym chosen by the student and used in each of the surveys.

Results: The facility of completing the surveys made the percentage of students involved very high. This has allowed us to follow up the students both globally and individually, studying also the evolution of the student in each of the thematic blocks.

Conclusions: With all this, we can get to know the perspective of the students about these subjects and improve key points of our teaching.

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WHAT'S BEYOND A NAME: THE IMPORTANCE OF BEING FIBROBLAST

Francisco José Sáez.

Department of Cell Biology and Histology, School of Medicine and Nursing, The University of the Basque Country UPV/EHU, Spain.

Introduction: The inmature and the final mature cell types of the tissues are denoted respectively using the suffixes *-blast* (from the ancient greek *βλαστός blastós*, that means germ or source) and *-cyte* (from *κύτος* kýtos, that means container or cell). In contrast with this general rule, the mature cell of the connective tissue is usually named fibroblast, instead of fibrocyte. However, some authors name fibroblast to the cell that actively synthesizes extracellular matrix (found in the connective tissue in development or repair), and fibrocyte to the cell that has a poor synthetic activity (found mainly in the mature connective tissue).
The aim of this work was to analyze the use of both terms.

Material and Methods: I searched the words fibroblast and fibrocyte in internet search engines and in scientific databases (Google, Google Schoolar, PubMed, Scopus and Ovid Medline) to count the number of results. In addition, I analyzed the use of both terms in 27 textbooks and histology atlases.

Results: The search in internet engines and scientific databases of the terms fibroblast and fibrocyte showed that the term fibroblast is used in a much larger proportion.
Only nine of the 27 books analyzed discriminate between the inmature form, or fibroblast, and the differentiated cell, or fibrocyte. The remaining 18 textbooks and atlases use the term fibroblast to designate both the inactive and the active cells. In four of these books the use by some authors of the term fibrocyte is mentioned, but two of them refuse the term.

Conclusions: The use of two terms, fibroblast and fibrocyte, to designate the inactive and the active form of the connective tissue cell, is less usual than the use of the term fibroblast to name both forms of the cell.
I suggest using the term fibroblast to designate both forms of the cell, following the explanation of Bloom and Fawcett (1983), who remind us that the term fibroblast was first used with the meaning of "fiber-maker".

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PRACTICAL TEACHING OF HISTOLOGY USING NFC TECHNOLOGY.

Ignacio Jimena ,[1,2,3], Miguel Ángel Gómez-Luque [1,2], José Peña [1,2,3], Irene Luque-Ruiz [4], Miguel ÁngelGómez-Nieto [4]

[1]Department Morphological Sciences, Section of Histology, Faculty of Medicine and Nursing, University of Córdoba, Spain.
[2]Research Group in Muscle Regeneration, University of Córdoba, Spain.
[3]Maimónides Institute for Biomedical Research IMIBIC, Reina Sofia University Hospital, University of Cordoba. Spain.
[4]Department of Computing and Numerical Analysis, School of Engineering Sciences, University of Córdoba, Spain

Introduction: Over the last years, many electronic teaching tools have complemented lectures and practical sessions in Medical Histology. This subject has undergone a change in the digital use of its contents and didactic methods based on virtual microscope, e-learning portals, recorded lecture videos, online learning modules, Web applications, etc. Medical Histology requires visual learning that obliges students to be trained to analyze microscopic images. This study describes a mobile device solution based on the use of Near Field Communication technology for the practical teaching in Medical Histology.

Material and Methods: We have designed and developed a Web solution integrating Near Field Communication (NFC) technology called HistoNFC. In laboratory sessions students interact with histological samples with an associated NFC chip by means of their Smartphone. During the development of practical sessions 121 students were registered in the system. The histological slides used to carry out the experiment corresponding to organs in the digestive tract. Prior to the development of the experiment we uploaded to the HistoNFC database the most relevant information about basic concepts that students need to know about tissues and organs, including those used in the slides for the experiment.

Results: An anonymous survey was conducted and consisted of 22 items divided into two sections destined, respectively, for the evaluation on teaching aspects and assisted-learning, and HistoNFC as a technological support and use of HistoNFC. The results obtained were very favourable for both sections. The students valued HistoNFC as an easy to use, attractive and versatile tool. They also considered that HistoNFC can be favourable in the theoretical and practical learning process of the Medical Histology.

Conclusions: The students have at their disposal an appropriate teaching support in their Medical Histology practice training. HistoNFC has also shown itself to be a powerful tool for teachers since it can be used for learning tool, questionnaires and assessment of students.

EPONYM TERMINOLOGICAL PERSISTENCE IN THE FIELD OF NEUROHYSTOLOGY: NEURONES OF PURKINJE

Jorge E Duque Parra [1,2]. Genaro Morales Parra [2]. John Barco Ríos [1]

[1] Departamento de Ciencias Básicas, Facultad de Ciencias para la Salud, Programa de Medicina, Universidad de Caldas, Colombia.
[2] Departamento de Ciencias Básicas, Facultad de Ciencias para la Salud, Programa de Medicina, Universidad de Manizales, Colombia.

Introduction: the use of eponyms remains as a frequently practice used by histologists, pathologists, clinicians and academics in the field of health including histological teaching; to refer various components in neurohistology such as Purkinje neurons. This eponym is even used in the *Terminologia Anatomica*, but the eponyms terms are not accepted. Although eponyms played a linguistic role in scientific terminology, the purpose of an international standard nomenclature or terminology in a scientific field is to ensure clear communication without eponyms. Eponyms are now considered obsolete because it have no descriptive or informative value on the histological element under consideration, but are still used traditionally without facilitating learning, without determining a cognitive process that grasp the form or function that goes hand in hand with the scientific method.

Materials and Methods: it was consulted various bibliographical sources referring to the terms used to designate the larger nerve cells of the cerebellum, using Purkinje neurons and Purkinje cell stratum as key words to see their presentation number and proposals linked to a terminological change.

Results: Purkinje, purkinjenoma, purkinjense stratum, Purkinje cell layer, pyriform cerebellar neurons, magnum cerebellar neurons, cerebellar gabaergic cells and estratum purkinjenase were found in relation to neurons in the intermediate layer of the cerebellar cortex. Only two of 1000 peer reviewed articles linked to cerebellum cells approaches its structural and functional description.

Conclusion: Purkinje´s eponym for cerebellar neuron, purkinjense stratum for the intermediate layer of the cerebellar cortex or purkinjenoma in this pathological connotation, is used in 99.8% of the professional work of the field of health, but the term should be used and adopted pyriform cells -pear shaped- or magnum cells -the largest- of the cerebellum, being dismissed gabaergic because there are other cerebellar neurons that use this neurotransmitter.

UTILITY OF MICROSCOPY PRACTICE NOTEBOOK IN HISTOLOGY LEARNING

María Sancho-Tello, José J. Martín de Llano, Manuel Mata, Lara Milián, Pilar Molina, Carmina Montoliu, Rosa Noguera, Santiago Peydró, Amparo Ruiz-Saurí, Irene Tadeo, Carmen Carda.

Department of Pathology, Faculty of Medicine and Odontology, Universitat de València, and Biomedical Research Institute (INCLIVA), Valencia, Spain.

Introduction: In the learning of Histology, making a notebook of microscopy practices, where students draw the tissues and organs they observe, reinforces the knowledge they are achieving. In the present study we compare if the notebook made in the same practice lab or at home, modifies the student's marks.

Material and Methods: We analyzed the marks obtained by the students of the subjects “General Histology” and “Histology”, taught during the first year of the degrees in Medicine and Odontology, respectively, throughout 2 consecutive years (2015-16 and 2016-17). We only studied the marks obtained by non-repeater students presented in the first call. During 2015-16, the students observed the histological slides in the practice lab and presented their notebook on the day of the exam, so they had the opportunity to complete their work at home. However, during 2016-17, students had to present the work at the end of each practice, being forced to better use their time at the practice lab. The marks used for this study were those from: the practice notebook (maximum 0.5 points), the exam of microscopy slides (maximum 1) and the test-type exam of practices (maximun 0.8) and theory (maximum 4). A statistical study was conducted.

Results: In both years analyzed, the presentation of the notebook was voluntary, but the percentage of students who presented it was around 64% in 2015-16, while it was 100% in 2016-17, in both degrees. Comparing both years, there were no significant differences in the average mark obtained in any item analyzed, but in 2016-17 we did observe a slight increase in the sum of the notebook mark plus microscopy slides exam, since, due to the increase in students presenting the notebook, the notebook mean mark increased slightly.

Conclusions: The presentation of the notebook at the end of each practice does not vary the marks obtained by the students, therefore we propose to evaluate the notebook worked at the lab, since it encourages all students to present it and at the same time saves them time at home, besides inducing students to use to the maximum the time in the practice lab.

THE ROLE OF TEACHING LEARNING TECHNIQUES IN HEALTH SCIENCES CURRICULUM

Martín-Piedra MA[1], Jaimes-Parra BD[1], Campos F[1], Durand-Herrera D[1], Ruyffelaert A[2], Santisteban-Espejo A[3], Alaminos M[1], Garzón I[1]

[1] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[2] Department of Linguistics, Ghent University, Belgium and Department of Didactics of Language and Literature, University of Granada, Spain
[3] Servicio de Hematología y Hemoterapia, Hospital Universitario Puerta del Mar de Cádiz

Introduction: Regulated teaching is considered the central axis of formation in Health Sciences curricula for the learning of knowledge and the acquirement of required abilities for an adequate clinical practice. Yet, there is a need of complementary formative activities for an optimal professional development in these degrees. The use of complementary activities in regulated teaching could be an appropriate tool for the acquisition of knowledge and competences, especially in degrees with high requirements of professional abilities as is the case of the Degree in Nursery.

Material and methods: A formation workshop on histologic methodology and histology applied to Nursery was performed with theoretical and practical sessions during the academic course 2016-17. The aim and scope of the workshop was the microscopical identification of human tissues related to the nurse practice, as well as learning on the most used techniques for conservation and histological analysis of tissue samples obtained in clinical practice. Students' satisfaction and perception on the workshop was evaluated at the end of the program by using a scale ranging from 0 to 10 by using a telematic survey.

Results: A virtual microscopy application allowed the students to properly visualize and identify the main structures related to histology in nursery and their future clinical practice. Average satisfaction of the students was 7.30.

Conclusions: These complementary activities are well evaluated by the students and could contribute to a more practical formation of the students, especially in specialties whose formation in histology at the pregraduate level is very limited.

SMARTPHONE AUDIOVISUAL CLIPS AS A REPETITION STRATEGY TOOL FOR LEARNING HISTOLOGY

Martín-Piedra MA[1], Carriel V[1], Campos-Sánchez A[1], Alaminos M[1], Rodríguez IA[1,2], García JM[1], Garzón I[1], Sánchez-Quevedo MC[1]

[1] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[2] Cátedra B de Histología, Facultad de Odontología, National University of Cordoba, Argentine

Introduction: In practical histology, observation, description, diagnostic, correlation and problem solving are competences that students should reach. For this purpose, microscopic slides are used and analyzed by each student in a self-learning discovery process. Repetition strategies may be used to fix and consolidate information and competences, and different approaches have been used, including virtual microscopy, repeated sessions in the microscopy laboratory, microscopy atlases, etc. The objective of this study is to design a novel strategy of repetition based on audiovisual clips that are sent to the students using a smartphone system.

Material and methods: First, 10 histological images corresponding to basic tissues that the students should know -bone, cartilage, muscle, epithelium, etc.- were obtained in a light microscope using 100x, 400x and 600x amplification. Then, a 3-minute of duration audiovisual clip was elaborated including a 3 levels sequence. The first step is labeled as “observe” and includes a naked histological image of the problem tissue. The second step is labeled as “identify” and includes the same image with specific structures being highlighted with arrows to be identified by the students. The third step is labeled “diagnose” and structures previously highlighted with arrows are labeled with the correct diagnosis after a short period of time.

Results: After each practical histology session in the microscopy laboratory, students receive the audiovisual clip corresponding to that session by smartphone at different periods of time to reinforce the information received and the competences acquired during the presential session. This pilot study has shown that perception of the students is highly positive, especially if the audiovisual clips are sent close to the examination period. Perception of teachers is also very positive because the reinforcement process is especially addressed to the main competences that students should reach.

Conclusions: Design and results of the study support the use of smartphone audiovisual clips as a repetition strategy tool for learning histology.

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IDENTIFICATION OF THRESHOLD CONCEPTS IN TISSUE ENGINEERING THROUGH A BIBLIOMETRICAL ANALYSIS OF RESEARCH AREAS AND KEYWORDS

Martín-Piedra MA[1], Santisteban-Espejo A[2], Ruyffelaert A[3], Carriel V[1], Campos F[1], Crespo PV[1], Campos A[1], Alaminos M[1]

[1] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA
[2] Servicio de Hematología y Hemoterapia, Hospital Universitario Puerta del Mar de Cádiz
[3] Department of Linguistics, Ghent University, Belgium and Department of Didactics of Language and Literature, University of Granada, Spain

Introduction: Tissue engineering (TE) is a developing research area that has more than 30 years since it was introduced by Wolter and Meyer in 1984. Since then, it has been described a widespread of clinical applications and due to the accumulation of knowledge, TE is changing its status from emerging to a consolidated area. For the first time, TE teaching is beginning and, thus, it is necessary to configure optimal learning strategies for a better knowledge by health science students. The aim of this work is to identify threshold concepts -terms once they are understood mean a significant comprehension of a matter- through a bibliometric analysis of the corpus of literature in TE from 1991 to nowadays.

Material and methods: Journal articles related to TE were retrieved by searching ("TISSUE ENGINEER*" or "TISSUE-ENGINEER*") as topic on Web of Science SCI-Expanded collection, excluding reviews, book chapters, meeting abstracts and proceeding papers, from 1991 to 2016. Once obtained, all the journal articles were assessed by the following criteria: year of publication, research area, author keywords and keywords plus.

Results: Total number of journal articles related to TE have increased progressively each year. Even, document distribution by year of publication shows a close adjust to an exponential model ($R^2 = 0.9448$). Recently, publication growth is changing from exponential to linear, suggesting a consolidation and knowledge archive of TE. Material Science and Engineering are the most prolific areas publishing 55% of global production, followed by Life Sciences & Biomedicine (27%) and Physical Sciences (18%). These results are in accordance with keywords analysis, as terms as "scaffold", "electrospinning", "hydrogel" or "biomaterials" appears among the 10 most-repeated words.

Conclusions: Bibliometric analysis performed on literature related with a research area could be used for identification of the most important and significant concepts (threshold concepts) in that subject. This bibliometric approach suppose a feasible tool in order to configure learning strategies of TE. An appropriate understanding of terms like "Engineering", "Cellular Biology", "Cells", "Scaffold", "Hydrogel", "Tissue", "Biocompatibility", "Differentiation", "Porosity" or "Regeneration" plays a key role in the comprehension of TE as a subject.

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USE OF WIKIS AS A TEACHING METHOD IN UNIVERSITY: EXPERIMENTAL HISTOLOGY, CHEMISTRY, ENGINEERING AND AUDIOVISUAL COMMUNICATION DEGREES.

Miguel A Ortega[1], Ana Díez[2], Pilar García[3], Rafael Cambralla[3], Natalio García-Honduvilla[1,4]

[1]Departments of Medicine and Medical Specialities, Faculty of Medicine and Health Sciences, University of Alcalá, Alcalá de Henares. Networking Biomedical Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain. [2]Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering, Faculty of Biology, Chemistry and Environmental Sciences, University of Alcalá, Alcalá de Henares, Madrid, Spain. [3]Department of Signal Theory and Communications, Polytechnic School, University of Alcalá, Alcalá de Henares, Madrid, Spain.[5]University Center of Defense of Madrid (CUD-ACD), Spain.

Introduction: Wikis are known more than 20 years ago. They belong to the social software, together with blogs and forums. A Wiki is free on-line software which provides a collaborative environment for generating knowledge, where each user can add, edit and publish content in order to correct, improve or update the website.

Material and Methods: We have used Wikis as a teaching methodology in three subjects of different degrees during the academic years 15/16 and 16/17: 'Basic Operations of Laboratory' in experimental sciences, 'Digital Communications' in Engineering and 'Technological Resources in Audiovisual' in Audiovisual Communication. The first used the Wikispaces platform, meanwhile the others the Virtual Classroom. The main goals for using Wikis in teaching can be summarized as: teacher-student direct communication lines out of the classroom, feedback from teacher to students, an instrument of evaluation, encourage students to work on their subject (Internet tool as a persuasive method with new technologies far away from traditional teaching practice), motivate students to do their own investigation, autonomy and self-government of their process of knowledge. The proposed activity for experimental sciences students was a bilingual glossary of technical terms. In 'Digital Communications' the teacher proposed exercises to solve during 15/16 and a writing work for 16/17. Students of Audiovisual Communication also worked on a writing task related to the subject. All of them were voluntary activities, and only in the Audiovisual Communication degree contributed to the final mark.

Results: Only the students most committed with the subjects were involved in the Wikis. Students are motivated to improve their marks. When they obtained an extra mark, the participation level reached 22.8%. The opposite occurred when the activity did not improve their marks (5.2%). Students prefer to implement practical exercises in the Wiki rather than writing about the subject. Students that took part in the Wikis got marks within the top 53%.

Conclusions: This study contributes to improve their teaching and to exchange experiences about their goals in the educational work.

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IDENTIFICATION OF COGNITIVE LEVELS OF TISSUE ENGINEERING THRESHOLD CONCEPTS IN POSTGRADUATE MEDICAL DOCTORS

Sola M[1], Santisteban-Espejo A[2], Martín-Piedra MA[3], Campos F[3], Carriel V[3], Crespo PV[3], Campos A[3], Alaminos M[3]

[1] Family Medicine Unit, University Hospital of Jaén, Spain
[2] Servicio de Hematología y Hemoterapia, Hospital Universitario Puerta del Mar de Cádiz
[3] Tissue Engineering Group, Department of Histology, Faculty of Medicine, University of Granada, Spain and Instituto de Investigación Biosanitaria ibs.GRANADA

Introduction: Along with cell therapy and gene therapy, tissue engineering is considered as an advanced therapy by the European Medicines Agency (EMA). Knowledge of basic threshold concepts in tissue engineering should be present in the formation of medical doctors involved in primary care medicine, due to the key role of these professionals in health education related to the correct use of these therapies. However, no specific formation in tissue engineering is present in the core curriculum of most medical schools.
The present study is focused on the identification of the cognitive levels of tissue engineering threshold concepts in medical postgraduates enrolled in a training specialization program in primary care medicine.

Material and methods: A questionnaire was used to evaluate the cognitive knowledge level of medical postgraduates enrolled in a training specialization program in primary care medicine. This questionnaire includes 30 items related to advanced therapies, artificial tissues, cell and tissue bases of the human body, novel medical products and regulatory frame. Each question is rated from 1 to 5 using a Likert-like scale.

Results: Our results demonstrate that the highest levels of knowledge in tissue engineering threshold concepts are those related with the cell and tissue bases of the human body (3.86±1.34), which is closely related with the preclinical formation in medical schools. In contrast, the lowest values were found for concepts connected to the regulatory frame established in the European Union for this type of therapies (2.05±1.28). Differences were statistically significant ($p>0.001$). The regulatory frame and items related to advanced therapies, artificial tissues and novel medical products are scarcely present in the curriculum of medical students.

Conclusions: Diagnostic evaluation performed in this type of professionals demonstrates the need of implementing formation programs in advanced therapies and, especially, in tissue engineering not only in pregraduate students, but also in professionals involved in health education.

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COMPARISON OF PERFORMANCE IN PRACTICAL EXAM WHEN USING VIRTUAL VS. REAL LIGHT MICROSCOPE IN PRACTICAL LESSONS OF MEDICAL HISTOLOGY

Sara Gayoso, José María Fernández, Manuel J. Gayoso, Fco. Javier Agudo, Manuel Garrosa.

Department of Cell Biology, Histology and Pharmacology, Faculty of Medicine and INCYL, University of Valladolid, Valladolid, Spain.

Introduction: Practical lessons of Medical Histology have been traditionally based on observations through light microscope. New technologies offer now much higher diversity of opportunities to implement in these lessons in order to improve students' performance. We have compared students' achievements in the identification of histological structures, comparing the use of virtual microscope instead of the classical lesson in the course of Medical Histology.

Material and Methods: Medical students of the second semester taking Medical Histology were aleatory divided in two groups. First group was taught in the classical manner, i.e., using the real microscope only, whereas the second group did not perform observations through the microscope, but studied histological slides by means of different virtual microscopes available on the Internet. The one-hour lesson started with an explanation of about 10-15 minutes by the lecturer on the structures to observe and continued with the personal work helped by the lecturer in both groups. Nevertheless, in the group using the virtual microscope, teams of four students were able to discuss the images on the computer, therefore working in a cooperative manner. Both groups received the same final practical exam consisting in the identification of the organ in 5 real histological slides plus 5 virtual ones, the students being examined in two rounds, on different sets of slides/images.

Results: Students in the real-microscope group answered correctly 3,05±0,95 real slides and 4,28±0,95 virtual images, whereas the virtual group succeeded 3,33±1,27 on the real slides and 4,32±1,05 on the virtual ones, obtaining a total grade mean 7,33±1,36 in the real-microscope group and 7,65±1,67 in the virtual-microscope group. The performance of students having taken the exam in the first round was 7,35±1,35 whereas students in the second round obtained 7,54±1,61 total grade. Student t test revealed no significant differences between the grades obtained by the two groups of teaching nor by the two different rounds with respect to this total grade. However, when the two rounds are compared paying attention to the real slides answers and to the virtual ones separately, differences ($P<0,001$) appeared for both the real slides (2,82±1,04 in 1st round vs. 3,45±1,06 in the 2nd) and for the virtual images (4,54±0,78 in the 1st vs. 4,10±1,10 in the 2nd). No significant differences appeared when contrasted across the two groups of teaching regarding rounds. In addition, regardless rounds, t-test revealed difference ($P<0,001$) between real vs. virtual slides both in the real-microscope group (-1,23±1,34) and in the virtual-microscope group (-0,98±1,64) as well as when considered both groups together (-1,13±1,46), resulting a grade mean of 3,16±1,09 for the real slides and 4,30±0,99 for the virtual images.

Conclusions: The use of virtual microscope together with cooperative work consistently rendered higher grades when compared to the use of real microscope only, but these differences did not reach statistical significance. Students perform more successfully when the practical exam consist of virtual images than when they have to identify the histological structure observing through the real light microscope.

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DIDACTIC SEQUENCE FOR THE MEANINGFUL LEARNING OF HISTOLOGY IN THE CURRICULAR MAP OF THE HYDROBIOLOGIST

Xochitl Guzmán García[1], Irma Hernández Calderas[1], Dalia I. Luis Hernández[1], Felipe de J. Muñoz González[1], Jonathan V.J. Hernández Torres[1], J. Roberto Jerónimo-Juárez[1].

[1]Departamento de Hidrobiología. División de Ciencias Biológicas y de la Salud. Laboratorio de Ecotoxicología, Histopatología. Universidad Autónoma Metropolitana Unidad Iztapalapa. Ciudad de México, México.

Introduction: Histology can be a valuable tool in the training of Hydrobiology undergraduate students at UAM. Unfortunately within the curricular map of that degree, the corresponding subject is not found. In the teaching of experimental sciences it is proposed the use of didactic sequences with a constructivist approach for the development of procedural skills. The aim of this work was to generate and apply a didactic sequence to Hydrobiology undergraduate students in order to contribute to the meaningful learning of histology.

Material and Methods: A didactic sequence was generated for five sessions, implemented during 2015 and 2017 to five groups of UAM Hydrobiology. The contents reviewed in each session were: general concepts of histology, histological technique, collection and processing of samples, and observation and analysis of basic tissues. The apprenticeships were evaluated by means of diagnostic tests, checklists and questionnaires. Videos, databases and field trips were used as didactic resources. The results of the learning domain were expressed in percentages.

Results: On average the implementation of the didactic sequence showed the following results. After the diagnostic evaluation, 45% of the students demonstrated basic knowledge on histology. 80% recognized the importance of histological technique in the assessment of aquatic resources. Subsequently, the checklist indicated that 90% assimilated conceptual learning and procedural skills on tissue processing. Of the students evaluated, 80% complied the criteria established in the checklist during the field trip. 90% can theoretically recognize basic tissues, but only 70% recognize them in practice. The final evaluation, shows that 80% of the students dominate the contents reviewed in the didactic sequence.

Conclusion: The applied didactic sequence showed that between 80% and 90% of the students acquire the development of theoretical-practical skills in the teaching of histology, achieving a meaningful learning of the same.

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QUANTITATIVE ANALYSIS OF SURVIVAL OF DOPAMINERGIC NEURONS, ASTROCYTES, OLIGODENDROCYTES AND MICROGLIA COTRANSPLANTED TO A PARKINSON'S DISEASE MODEL.

Deisy Hernandez-Zuñiga[1], Gabriela Camacho-Cortes[2], Jose Rafael Godinez-Fernandez[3], Anabel Jimenez-Anguiano[4], Nohra E. Beltran[5], Mario Garcia-Lorenzana[4].

[1] Doctorado en Biologia Experimental.
[2] Licenciatura en Biologia Experimental.
[3] Departamento de Ingeniería Electrica.
[4] Departamento de Biologia de la Reproducción. Universidad Autonoma Metropolitana, Iztapalapa. Avenida San Rafael Atlixco 186, Colonia Vicentina, 09340 Ciudad de Mexico.
[5] Departamento de Procesos y Tecnologia, Universidad Autonoma Metropolitana, Cuajimalpa. Av. Vasco de Quiroga 4871, Colonia Santa Fe, 05300 Ciudad de Mexico.

Introduction. Parkinson's disease (PD) is the second most common neurodegenerative disease and the most frequent movement disorder. It is generated by dopamine in Basal Ganglia decrease, due to dopaminergic neurons (DN) of substantia nigra death. The goal of cell therapy is to replace dead cells with new functional cells. The motor recovery of PD models after transplant surgery is always associated with the presence of neurons, however the cultures used contain only 8-22% of dopaminergic neurons. Therefore, it seems reasonable to evaluate the survival of DN and glial cells.

Material and Methods. Female Wistar rats (200 g) were unilaterally injured with 4 µl of 6-OHDA neurotoxin into the medial forebrain bundle and co-transplanted with neurons and glial cells in the striatum. The primary coculture cells used for the cotransplant were stained with Vibran DiI fluorescent dye (Molecular probes) before the surgery. The motor behavior of the PD model was evaluated with the rotation test in response to apomorphine, 4 weeks after injury surgery and 2 weeks after co-transplantation surgery. Finally the animals were sacrificed, fixed (irrigation-intravascular / paraformaldehyde 4%), and brains were processed for immunofluorescences. The antibodies used were anti-TH/GFAP/Oligo/CD68. The secondary antibodies used were Alexa 488 anti-mouse/sheep and all nuclei were stained with DAPI. From each group 2 cuts were analyzed per brain, 3 fields of each cut were randomly selected to perform the corresponding counts with an original x400 increase. The material was analyzed with the divisional confocal microscope Carl Zeiss (LSM 780 NLO) at UAM-Iztapalapa.

Results. At deposit sites, we found dopaminergic neurons (TH+/Vibran DiI+), astrocytes (GFAP+/ Vibrant DiI+), oligodendrocytes (Oligo+/ Vibrant DiI+), and microglia (CD68+/Vibrant DiI+). The transplants quantitative histological analysis shows that astrocyte is the cell type with the greatest survival ($p<0.01$) compared with other cell types.

Conclusion. The cell type with the greatest survival in the cotransplant is the astrocyte followed by: dopaminergic neurons, oligodendrocytes and microgliocytes. Motor recovery of injured individuals should not only be associated with the survival of dopaminergic neurons but also should be due to the survival of glial cells.

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CHORIONIC TERMINAL VILLI IN NORMAL PLACENTAS IN ADVANCED MATERNAL AGE. A MICROSCOPY STUDY

Jorge Henríquez[1], Aylin Andrade[1], Rene Cornejo[1].

[1]Faculty of Medicine, Universidad de La Frontera, Temuco, Chile

Introduction: Pregnancy in advanced ages, is a condition that has increased in recent years worldwide. National figures indicate that this increase has been around 10% of all pregnancies in 1990 and 16% in 2006. (MINSAL, 2006). Due to this increase of pregnancies in advanced age and its effect in the presence of certain maternal fetal pathologies, it is that this study presents special relevance. The objective of this study was to determine the morphological characteristics of the terminal chorior villi of normal placentae in advanced maternal age.

Material and Method: Samples were obtained from 5 normal placentas of women whose ages correspond to 35 or more years, and 5 normal placentas of women between 18 and 29 years of age, without known maternal-fetal pathology. From each placenta were obtained 2 samples from opposite parabasal regions and 1 from the central parabasal region, with a total of 15 samples each group. They were processed according to transmission electron microscopy technique, studied and micrographed on a Phillips 300 microscope and obtaining images with an increase of up to 7000X. In these the variables to be measured were: area and thickness of the syncytiotrophoblast, percentage of samples in which the cytotrophoblast is found, thickness of vasculature membranes. For the quantitative analysis of group comparison, the Student's t-test was applied.

Results: In all the studied parameters it was observed that there are notable differences between both age groups, indicating that the results of the analyzed variables were homogeneous within each placenta.

Conclusions: These results suggest that there is a better maternal-fetal exchange in placentas of young women.

IMMUNOHISTOCHEMICAL EXPRESSION OF CNPase MOTOR FIBER MARKER IN TRANSVERSAL CUTS OF HUMAN BRACHIAL PLEXUS.

Juan Carlos Torrez A[1], Karen Abarzua S[2], Nubia Riquelme Z[3], Francisco X Torrez A[4]

[1] Medical Surgeon, Anatomy Professor, Universidad de La Frontera, Candidate program of Doctorate in Morphological Sciences Faculty of Medicine, Temuco Chile.
[2] Junior Biochemist, Research Laboratory in Animal Biotechnology UFRO Temuco Chile.
[3] Medical Surgeon, Medical Legal Service, Temuco, Chile.
[4] Medical degree graduate, Universidad Mayor, Temuco, Chile.

Introduction: Peripheral nerves consist of individual nerve fibers that can be motor, sympathetic sensory and myelinated or non-myelinated. CNPase is an enzyme present at high levels in the central and peripheral nervous system. CNPase is considered a marker for myelin forming cells. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to human CNPase.

Material and Method: The objective of the present study was to experiment new techniques for the identification of motor fibers in human brachial plexus. The study was performed in 12 brachial plexus, obtained from 6 fresh male adult cadavers, between 20 and 90 years of age. The pieces were obtained from the Legal Medical Service unit. Samples were fixed in 4% paraformaldehyde for 48 hours. Following assembly of the samples, the evaluation was performed through immunohistochemistry techniques using RTU kit. In the immunohistochemistry, we used the ABC technique with diaminobenzidine development, while for the immunofluorescence the indirect technique with fluorophore tag Alexa Fluor 633 was used. For both we used the antibody CNPasa in concentration of 1/250.

Result: The fibers of peripheral nervous tissue presented staining in their fascicular and non-fascicular components. The motor fibers present the specific immunohistochemical mark distributed in the fascicles in heterogeneous form. The expression of the marker does not present background or non-specificity, and highlights staining only in the motor fibers, which demonstrates that the antibody used against the CNP protein was specific for motor fibers. No positivity was detected in the negative controls.

Conclusions: The proven technique is of great help in identifying motor fibers of the human brachial plexus.

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LEARNING HISTOLOGY THROUGH FILM FRAMES

Rosa Álvarez-Otero, Encarnación de Miguel, Mª Jesús I. Briones.

Faculty of Biology, University of Vigo, Vigo (Pontevedra), Spain.

Introduction: Advances in Histology are partly due to its interaction with other disciplines such as Physiology, Biochemistry, Genetics, etc. In the same way, in the academic field, improvements in learning methodologies have been obtained thanks to the incorporation of new technologies and internet and the use of resources such as movies. By using films, the students get actively involved in acquiring knowledge rather than being simple receivers of the concepts transmited by the teacher.

Material and Methods: Every academic year the authors of this contribution organize the activity known as "Cineforum of Biology" which consists of showing four carefully selected films in order to address different topics. Among them, those films dealing with any aspects that could bring the students closer to biosanitary research are typically included. The films are selected on the basis of two important criteria: their commercial success and their ability to promote discussions about the topic in question. In each session, a film is showed, then discussed with the organisers and finally, the students fill in a short questionnaire on the topics covered in each movie.

Results: Among the films used in this activity, "Un fueguito" (Ana Fraile, 2010) allows the analysis of the advances in immunology exemplified by the contribution of César Milstein to Cellular Biology and Histology. Undoubtedly the development of immunohistochemistry techniques is one of the main methods for *in situ* localization of molecular components at the tissue and subcellular levels, respecting the morphological context. Another good example is "Extraordinary Measures" (Tom Vaughan, 2010) a drama which describes the pathologies caused by the dysfunction of certain cellular organelles (peroxisomes, lysosomes, mitochondria) or by diseases associated with the cytoskeleton. Finally, "Concussion" (Peter Landesman, 2015) shows the histopathological characteristics of neurodegenerative diseases, and helps the students to become familiar with the classic neuroanatomical techniques and immunohistochemical stains as basic diagnostic tools.

Conclusions: Our experience reveals that the audiovisual language is a extremely good way by which students can apply theoretical knowledge to practical situations such as those raised in the film and the debate afterwards promotes critical thinking on the professional implications of their future careers.