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From Cell Biology to Tissue Engineering

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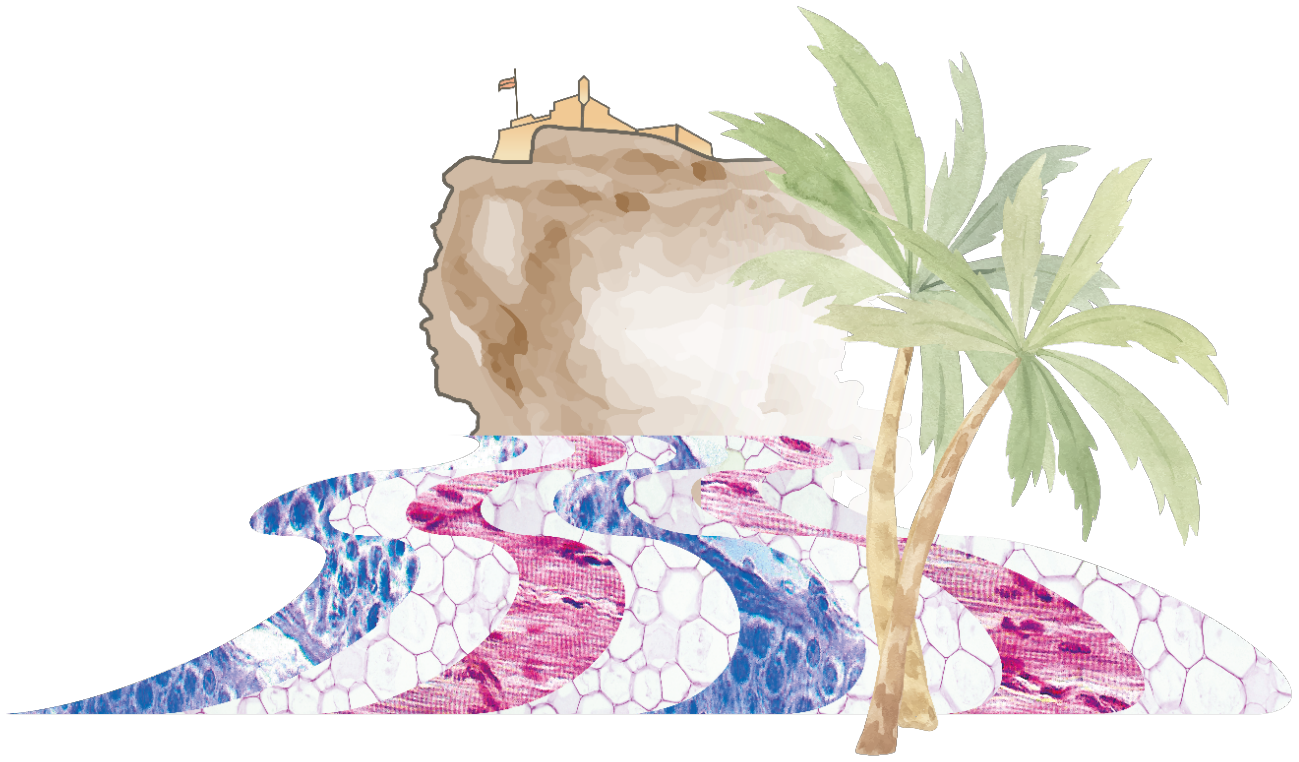

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Oral Presentations

Oral Presentations

Sessions of Wednesday 11th (12:30h -14:00h)

Oral 1 - Neurohistology

Moderators:
Yolanda Segovia Huertas
Hugo Ríos

Oral 2 - Tissue Engineering I

Moderators:
Gaskon Ibarretxe Bilbao
Fernando Campos Sánchez

Histology and Histopathology
From Cell Biology to Tissue Engineering

C01 - Dimethyl fumarate ameliorates both gray and white matter injury and stimulates cell proliferation after neonatal hypoxic-ischemic brain injury in rats

Alart J.A.[1], Chillida M.[1], Beldarrain G.[1], Herrero de la Parte B.[2], Alonso-Varona A.[1], Hilario E.[1], Álvarez A.[1] & Alonso-Alconada D.[1]

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Introduction: Birth-related disorders, such as perinatal hypoxic-ischemia (HI), are one of the majorcauses of death and childhood disability worldwide. Among these injuries, hypoxic-ischemic encephalopathy has a high prevalence, being the cause of approximately one million neonatal deaths per year. Additionally, more than half of the survivors will present severe neurological sequelae such as cerebral palsy or epilepsy. The only clinically approved treatment is therapeutic hypothermia, which is partially effective, reducing the death rate by 12%. Using histological techniques, the objective of this study was to evaluate the antioxidant dimethyl fumarate (DMF) asa neuroprotective agent in both gray and white matter, as well as its neurogenic potential, after neonatal HI in rats.
Methods: On postnatal day 7 (PD7), fifty-one Sprague Dawley rats were randomly assigned to: i) HI (left common artery ligation + 150min 8%O_2/92%N_2; n=24); ii) HI + DMF (45mg/Kg; one i.p. doseimmediately after HI and six oral doses spread over the next 72 hours; n=13); iii) Sham (without HI;n=14). At PD14, animals were sacrificed for neuro-histological assessments: i) hematoxylin-eosin staining to evaluate global, cortical and hippocampal neuropathological score, and to analyze hemispheric and hippocampal infarct; ii) immunohistochemistry to assess white matter injury (by quantifying Myelin Basic Protein staining) and cell proliferation (by Ki67 in the neurogenic niche ofthe hippocampal subgranular zone). One-way ANOVA was performed and statistical differences considered if $p<0.05$.
Discussion & conclusions: Animals treated with DMF showed a significant improvement in all neuro-histological analyses when compared to non-treated pups. Thus, a better neuropathologicalscore was observed, together with reduced infarct in both hemispheres (p=0,0035) and hippocampus (p=0,0047). This neuroprotective effect was further extended to white matter, with reduced damage in cingulum (p=0,0008), external capsule (p=0,0001) and caudoputamen (p=0,0004), also followed by a higher proliferation index in the hippocampus compared to the HI group (p=0,0025). These results suggest that DMF is effective against hypoxic-ischemic brain injuryin neonatal rats, with both neuroprotective and neurogenic effects.

Acknowledgement: Basque Government (PIBA_2023_1_0022); Programa Investigo funded by EU-Next Generation EU (PIFINVE22/14); UPV/EHU (GIU21/054).

C02 - Interaction of TRPC1 and TRPC5 channels mediates inner retinal preservation during photoreceptor degeneration

Caminos, E.[1,2], López-López, S.[1,3], Cabanes-Sanchis, J.J.[1,2] and Martínez-GalánJ.R.[1,2]

[1] University of Castilla-La Mancha. Medical School of Albacete. [2] Institute of Biomedicine (IB-UCLM), Group of Neurohistology and Development. [3] Research Unit, Complejo Hospitalario Universitario de Albacete. Albacete, Spain

Introduction: Neurons and glial cells of rat retinas with photoreceptor degeneration have basal calcium levels above those found in healthy retinas [1]. These cells of the inner retina are the last to degenerate by retinitis pigmentosa (RP). Transient receptor potential canonical 1 and 5 (TRPC1,and TRPC5) channels are calcium channels with distinct expression profiles and opposite physiological implications in retinal and brain cells, where they can interact with STIM1, one of thecomponents of store-operated calcium (SOC) channels. While the TRPC1 channel mediates protective effects in neurodegenerative processes, TRPC5 promotes cell death in cases ofintraocular pressure and it is a negative regulator of axonal growth in retinal ganglion cells and rat hippocampal neurites. In addition, TRPC1 is a negative regulator of the TRPC5 channel. We studied here whether TRPC1 could be form heterodimers with TRPC5 and what effect this interaction could have in the survival of the inner retinal cells that are responsible for maintaining the activity in visual areas of the brain.
Methods: Retinas from P23H-1 transgenic rats, an experimental model widely used to study retinitispigmentosa (RP), and healthy rat retinas (Sprague Dawley rats, SD), were used to identify the cellular immunolocalization and the quantitative levels of TRPC1 and TRPC5 channels. In addition,functional interactions between both channels, TRPC1 and TRPC5, were analyzed by proximity ligation assays.
Results: While the TRPC1 channel is prominently distributed throughout the retina [2], TRPC5 is restricted to the inner retina. Furthermore, we detect higher relative levels of TRPC1 in advanced degenerating retinas, whereas TRPC5 and STIM1 levels do not change in healthy and P23H retinas. Co-localization and subsequent physical interaction between TRPC1 and TRPC5 are found in SD and P23H rat retinas. We detected an overlapping signal in the innermost retina of SD and P23H rat retinas, mainly localized in the ganglion cell layer and nerve fiber layer. In this retinal region, TRPC1 and TRPC5 channels physically interacted, and this interaction significantly increased as photoreceptors loss progressed.
Discussion and Conclusions: Both calcium channels may function as TRPC1/5 heterodimers in thehealthy and damaged inner rat retina. In addition, our results support that homomeric TRPC5 channels function in partnership with TRPC1 and STIM1 in the rat retina. Müller and retinal ganglion cells have the necessary machinery for these channels to be active, either as homomers or as heteromers. Thus, it is plausible to think that the increase in TRPC1/5 heteromers during the outerretinal degeneration slows down the degeneration of the inner retina in P23H rats, where ganglionand Müller cells survive long time after complete photoreceptor degeneration.

Supported by the University of Castilla-La Mancha (UCLM, Spain) and European RegionalDevelopment Fund (FEDER) (2022-GRIN-34203).

[1] Caminos E., Vaquero, C.F. and Martinez-Galan J.R. (2015). Relationship between rat retinal degenerationand potassium channel KCNQ5 expression. Exp. Eye Res. 131, 1-11. doi: 10.1016/j.exer.2014.12.009.
[2] Caminos E., Murillo-Martínez, M., García-Belando M., Cabanes-Sanchis J.J., Martinez-Galan J.R. (2023).Robust expression of the TRPC1 channel associated with photoreceptor loss in the rat retina. Exp. Eye Res.,236. doi: 10.1016/j.exer.2023.109655.

C03 - Methylene blue reduces ganglion cell death and electroretinogram distortion in a rat model of glaucoma.

Nakamura R.[1], Ciranna N.S.[1], Fernández J.C.[1], Peláez R.[2], López-Costa J.J. [1] Loidl C.F. [1], Martínez A.[2], Rey-Funes M. [1]

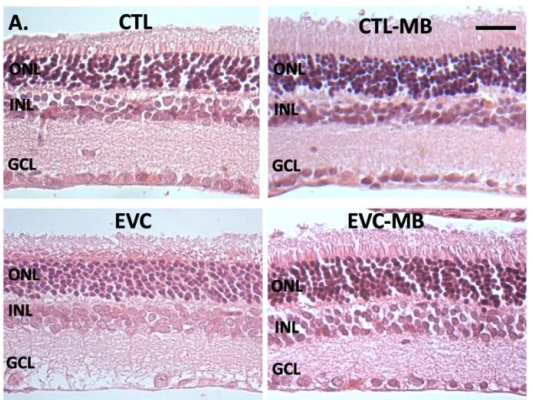
[1] Institute of Cell Biology & Neurosciences "Prof. E. De Robertis" (IBCN-CONICET), Faculty of Medicine, University of Buenos Aires, Buenos Aires, Argentina.
[2] Center for Biomedical Research of La Rioja (CIBIR), Logroño, Spain.

Introduction: Glaucoma is currently the leading cause of irreversible blindness worldwide. Eventhough several therapeutic approaches exist to treat this condition, both pharmacologically andsurgically, none of them addresses the real pathophysiological cause for blindness which is theneurodegenerative process in the retina. Given the evidence provided by our team on theneuroprotective role of methylene blue (MB) in other models of retinopathy, we propose MB as acandidate to reduce retinal damage in an episcleral vein cauterization (EVC) model of glaucoma.

Methods: EVC surgery was performed in male Wistar rats (n=38) unilaterally, with two episcleralveins being cauterized. Sham surgery was performed in the contralateral eye, which served ascontrol. Seven days post-surgery, half of the experimental animals started intraperitoneal MBtreatment (2mg/kg), twice a day for seven days. The other half received a placebo. Oscillatorypotentials (OP), scotopic (ERG) and pattern (PERG) electroretinography were performed at dayseven and fifteen post-surgery. At day fifteen, all animals were sacrificed. Eyes were enucleatedand subjected to morphological studies with hematoxylin & eosin (H&E).

Results and discussion: In EVC operated eyes, ERG showed amplitude reduction of the b-waveand OP alteration. In addition, PERG showed a significant reduction of N2-wave (equivalent to human N95 wave) and the implicit time was significantly prolonged. Histological evaluation showedretinal ganglion cells (RGC) loss and reduction of inner retinal (IR) thickness. No significant differences were observed between control (CTL) and CTL-MB groups. Our findings show that, compared with EVC group, EVC-MB had significant preservation of RGC and inner retinal thickness. Furthermore, the histoarchitectural damage produced by EVC surgery was greater in the peripheral retina. This finding is consistent with the current paradigm in glaucomatous retinal damage. Electroretinography findings of EVC-MB eyes showed significant waveform and amplitude preservation by MB. Both, ERG b-wave and PERG N2-wave values, account for inner retina neuroprotection and are consistent with our histological findings. It is of interest, that implicit time, the most sensitive parameter for RCG function evaluation, was significantly prolonged in both EVCand EVC-MB eyes. This finding implies that a certain level of cellular stress is still affecting RGC function, even in the presence of MB.

Conclusion: MB is an effective neuroprotective drug in preserving retinal structure and function, which is at risk under glaucomatous damage. This drug could be therefore representing a new therapeutic agent to prevent vision loss in glaucoma patients.



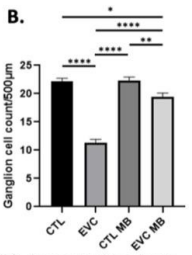


Figure 1. A- Representative histological images of the retina of animals of the 4 experimental groups, taken 15 days after surgery and stained with H&E. Three layers of the retina are labeled in the pictures for reference: Outer nuclear layer (ONL), inner nuclear layer (INL), and ganglion cell layer (GCL). Scale bar = 50µm. B- Bars represent the mean ± SEM of all samples (n = 3 animals per group, 7 measurements per animal). Asterisks represent statistically significant differences. *p<0.01; **p<0.005; ****p < 0.0001.

C04 - Methylene blue prevents retinal lesions in a model of experimental compression of the optic nerve.

Ciranna N.S.[1], Nakamura R.[1], Peláez R.[2], Paganelli A.[1], Fernández J.C.[1], López-Costa J.J.[1], Martínez A.[2], Rey-Funes M.E.[1], Loidl C.F.[1].

[1] Institute of Cell Biology & Neurosciences "Prof. E. De Robertis" (IBCN-CONICET), University of Buenos Aires, Buenos Aires, Argentina.
[2] Center for Biomedical Research of La Rioja (CIBIR), Logroño, Spain.

Introduction: Ocular and periocular traumatisms may result in loss of vision. In previous experiments of our team hypothermia treatment in retinas subjected to Intra-orbital optic nerve crush (IONC) has proven to reduce morphological and molecular alterations. On the other hand, our previous work also showed that methylene blue (MB) reduces morphological and electrophysiological retinal distortion caused by perinatal asphyxia. In the light of these findings, we propose MB as a neuroprotective strategy.

Materials & methods: IONC surgery or sham manipulation was applied to 45 days old Wistar rats.Sham surgery was performed in the contralateral eye which served as control. Intraperitoneal MB treatment (2mg/kg) was administered at immediate post-surgery, 6, 12 and 24 hours after surgery. Oscillatory potentials (OP) and scotopic (ERG) and pattern (PERG) electroretinography was performed twenty-one days post-surgery. After PERG evaluation, all animals were sacrificed. Eyeswere enucleated and retinal tissue was dissected for morphological studies with hematoxylin & eosin (H&E) stain.

Results and discussion: In IONC operated eyes, ERG showed a drastic amplitude reduction of the b-wave ($p < 0.0001$), a-wave ($p < 0.03$) and OP alteration ($p < 0.0001$). PERG of IONC operatedeyes showed a significant reduction of N2-wave ($p < 0.0001$), which is equivalent to human N95 wave. Histological evaluation showed a large decrease in the number of retinal ganglion cells ($p < 0.0001$). No significant differences were observed between control (CTL) and CTL-MB groups. Our findings show that, compared with IONC group, IONC-MB had significant preservation of retinal ganglion cells. Electroretinography findings of IONC-MB eyes showed significant waveform and amplitude preservation by MB. ERG b-wave values, account for inner retina neuroprotection and are consistent with our histological findings. N2 amplitude was significantly preserved by MB in IONC operated eyes. No significant differences of PERG's implicit time were observed among the experimental groups.

Conclusions: MB is an effective drug in preserving retinal histoarchitecture and electrophysiological function after IONC induced damage. It could therefore be used as a neuroprotective strategy to preserve retinal integrity an prevent visual loss in ocular and perioculartrauma patients.

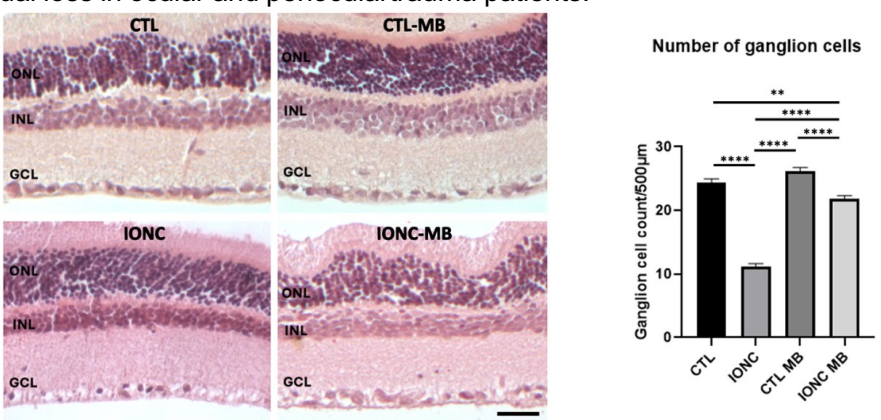


Figure 1. A-Representative histological images of the retina of animals of the 4 experimental groups, taken 21 days after surgery and stained with H&E. Three layers of the retina are labeled in the pictures for reference: outer nuclear layer (ONL), inner nuclear layer (INL), and ganglion cell layer (GCL). Scale bar = 50µm. B- Bars represent the mean ±SEM of all samples (n = 3 animals per group, 7 measurements per animal). Asterisks represent statistically significantdifferences. **p<0.01; ****p < 0.0001.

C05 - The role of blood vessels in the myelin alterations associated to alcohol abuse during adolescence.

García-Díaz B[1;2], Martinez-Padilla AB[2], Sanchez-Salido L[2], Sanchez-Marin L[3], Serrano A[3], Rodríguez de Fonseca F[2], Lopez-Villodres JA[1], Escamilla A[1], Sanchez- varo R[1] and Bermudez D[1].

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[2] UGC de Neurociencias Clínicas, Grupo de neuroinmunología y neuroinflamación, IBIMA Plataforma Bionand, Málaga, Spain.
3 Unidad de Gestión Clínica de Salud Mental, Hospital Regional Universitario de Málaga, 29010, Málaga, Spain.

Introduction: Drug addictions pose a serious problem in our society, being adolescence the periodin which its use generally begins, and alcohol the first drug of choice at this age. The exposure to drugs, such as alcohol, during adolescence leads to neurobiological and behavioural alterations, and adaptations that persist in the adulthood, often becoming permanent. These neuroadaptations constitute the base of the addictive process. Among other neurobiological alterations, chronic alcoholism is associated with reduced cerebral blood flow and cerebral metabolism. Vasculature stands out nowadays as an important facet for the migration of myelinating cells. Moreover, central myelinating cells (oligodendrocytes) demand high energy requirements (mainly glucose), which shall be supplied by the blood flow, to form myelin. Despite the already observed brain myelin alterations after alcohol abuse, and the mentioned blood flow and metabolic impairment due to alcoholism, so far no studies have demonstrated whether this cause-effect link between myelin and vasculature sets up the neurological bases of the cognitive impairment or behavioral alterations related to alcohol consumption.
Methods: Male Wistar rats were used to investigate the short- (24h post-administration) and long-term effects (2 weeks post-administration) of intermittent alcohol exposure (intragastricadministration of 3 g/kg ethanol; 4 days/week for 4 weeks during adolescence, from 31 to 55 postnatal days) on the histological myelin and blood vessel patterns in brain regions associated with cognitive impairment and emotional behavior (Hipocampus (Hpp) and medial Prefrontal Cortex (mPFC)). Rats from different groups at both time points were fixed by cardiac perfusion in 4% paraformaldehyde. After post-fixation, rat brains were sliced by vibratome (50 µm) incubated with primary antibodies against a myelin marker: myelin basic protein (MBP), an oligodendrocyte marker: pi form of glutathione-S-transferase (GST), and an endothelial cell marker: Glucose transporter 1 (Glut-1) overnight. Primary antibodies were revealed with fluorescent secondary antibodies (Alexa 647, Alexa 563 and Alexa 488). Finally, the slides were imaged by a Fluorescent Slide Scanner and image analysis was performed by Fiji open source platform.
Results & Discussion: We observed an altered myelin pattern in the Hpp of alcohol-treated rats with a significant increase in the myelinated fiber density but a reduction in the MBP staining at 24h after the last administration, which was normalized after 2 weeks. In this region, the vasculature density was not altered but it showed a significant decrease in the intensity of Glut1in the endothelial cells, suggesting a metabolic dysregulation of the Hpp environment. In contrast, the mPFC showed no changes in the myelin or vascular pattern at 24h, while 2 weeks after alcohol the density of GST+ cells (oligodendrocytes) was significantly reduced with no effect in the density of myelinated fiber or MBP staining, hinting an overactivation of the remaining oligodendrocytes to maintain the myelin pattern.
Conclusions: Chronic alcohol exposure alters the central myelin and blood vessel pattern in a timely and region specific manner. Although more studies are needed to establish the linkbetween both processes, these time- and region-specific alterations open the avenues to elucidate novel strategies to overcome the neurological bases of the pathogenesis of the addictive process.

This study has been funded by Instituto de Salud Carlos III (ISCIII) through the project "PI22-01141" and co-funded by the European Union.

C06 - Conditioned medium from H_2O_2-preconditioned mesenchymal cells ameliorates oxidative stress-induced damage to IEC-6 enterocytes.

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

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Introduction: Mesenchymal stem cells (MSCs), including adipose tissue-derived stem cells (ASCs),have gained traction in recent years for their potential for cell therapy. Aside from their differentiationpotential, it has been shown that most of the benefits of MSC therapy come from the bioactive factors they produce as paracrine factors delivered to medium (secretome). In fact, it has been indicated that conditioned media from ASCs show therapeutic effects like those achieved by ASCsthemselves. Besides, cell preconditioning with low doses of hydrogen peroxide (H_2O_2) can increasethe survival and adaptation of the cells to oxidative stress and boost their therapeutic potential, aswe have shown recently [1]. In this work, we have evaluated the therapeutic efficacy of conditionedmedium obtained from H_2O_2-preconditioned rat ASCs in a model of oxidative stress in IEC-6 enterocytes.

Methods: Adipose tissue was harvested from 4 male WAG/RijHsd rats. The ASC isolation protocolwas adapted from [2]. Briefly, the tissue was digested with collagenase 0.1% for 1 h at 37°C and filtered through a 70 µm cell strainer. The resulting cell suspension was centrifuged at 450g, the pellet was resuspended in ACK red blood cell lysis buffer, centrifuged again, resuspended in DMEMsupplemented with 10% FBS and 2% P/S, and seeded in 75 cm^2 flasks. 24 h later, the medium was replaced to select plastic-adherent cells. To confirm that the isolated cells were in fact MSCs,they were differentiated to bone, cartilage and adipose tissue by culturing them in commercial differentiation medium (StemPro™, Gibco) and stained with Alizarin Red, Alcian Blue or Oil Red, respectively. To obtain the conditioned media, the ASCs were seeded at a density of 10^5 cells/mL and cultured for 7 days in complete medium with 10 µM H_2O_2. Afterwards, the medium was replaced for serum-free medium for 48 h and collected, filter-sterilized and stored at -20°C. IEC-6 cells were seeded in 96-well plates at 10^4 cells/well and cultured in basal medium for 24 h to allowadhesion. The next day, to induce oxidative stress, the medium was removed and 100 µL of medium with 300 µM H_2O_2 were added and incubated for 1 h. The oxidation medium was replacedfor conditioned medium or serum-free medium as control. Intracellular ROS were quantified by loading the cells for 30 min with 20 µM DCF-DA after H_2O_2 exposure and measuring fluorescencein a plate reader 1 h after the addition of the conditioned medium. 24 h after the insult, cell viabilitywas quantified using resazurin-based PrestoBlue™ reagent and measuring fluorescence after 30 min, following the instructions from the manufacturer. The experiments were repeated thrice, and their mean and standard deviation was calculated.

Results: Firstly, the cells isolated from rat fat showed the typical fibroblast-like morphology of ASCs. Furthermore, Alizarin Red, Alcian Blue and Oil Red stains of cells cultured in osteogenic, chondrogenic and adipogenic differentiation media confirmed that these cells were, in fact, MSCs.Secondly, IEC-6 cells injured with 300 µM H_2O_2 showed a significant increase in ROS levels ($p<0.001$) and a decrease in cell viability ($p<0.01$). Treatment with ASC-conditioned medium resulted in a reduction of 76% in ROS levels with respect to the control ($p<0.001$), and an increaseof 85% in IEC-6 viability after 24 h ($p<0.01$).

Discussion & Conclusions: This study provides further evidence that H_2O_2 preconditioning enhances the therapeutic potential of ASCs, by showing that conditioned medium from these cellssignificantly reduces oxidative stress and increases survival of H_2O_2-challenged rat enterocytes.

[1] Burón M., Palomares T., Garrido-Pascual P., Herrero de la Parte B., García-Alonso I. and Alonso-Varona A. (2022). Conditioned Medium from H2O2-Preconditioned Human Adipose-Derived Stem Cells Ameliorates UVB-Induced Damage to Human Dermal Fibroblasts. Antioxidants 11, 2011-2029.

[2] Kilroy G., Dietrich M., Wu X., Gimble J.M. and Floyd Z.E. (2018). Isolation of Murine Adipose-Derived Stromal/Stem Cells for Adipogenic Differentiation or Flow Cytometry-Based Analysis. Adipose-Derived StemCells. Methods in Molecular Biology 1773, 137-146.

C07 - In Vitro Effect of D9-Tetrahydrocannabinol and Cannabidiol on a Tridimensional Model of Human Lung Cancer

Monleón-Guinot I.[1,2], Pérez V.[1], Millot M.[11], Sancho-Tello M.[1,2], Martín de Llano J.J.[1,2], Carda C.[1,2,3], Bravo-Baranda L.[1,2], Navarro-Esclapés I.[1], Llop-Miguel M.[1], Milián L.[1,2] and Mata M.[1,2,3]

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[2] Instituto de Investigación Biomédica INCLIVA, Valencia, Spain.
[3] CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN).

Introduction: Cannabinoid agonists can inhibit key factors in lung cancer, like cell proliferation or epithelial-mesenchymal transition (EMT) [1,2]. However, most of these studies are based on bidimensional *in vitro* models that do not consider the complexity of the tumor microenvironment (TME), which is involved in many characteristics of lung cancer, including metastasis and drug resistance. Cancer-associated fibroblasts (CAFs) play a crucial role in tumor progression through EMT as they can secrete different factors, including transforming growth factor beta (TGF-β).

Objective: In this work, we evaluate the effects of two cannabinoid agonists, ∆9- tetrahydrocannabinol (THC) and cannabidiol (CBD), on a tridimensional (3D) *in vitro* model of spheroids conformed by A549 cells and organoids of A549 in combination with CAFs or normal fibroblasts (NFs), which have been supplemented with TGF-β.

Methods: Organoids were generated using the hanging drop method and subsequently embeddedin a 3.9 mg/mL rat type I collagen hydrogel. They were then incubated in medium for 4 days. Afterthat time, they were supplemented or not with 5 ng/ml TGF-β, and treated or not with thecannabinoid mixture (THC + CBD, 10 µM each), after which they were further incubated for three days. Subsequently, the organoids were fixed in 4% paraformaldehyde. Immunofluorescence wasperformed to study cytokeratin filaments, F-actin was evaluated using rhodamine-conjugated phalloidin, and cell nuclei were stained with 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI).Finally, the organoids were analyzed using a confocal microscope.

Results: Immunofluorescence analysis revealed a consistent morphology in the A549 spheroids under normal conditions, with the exception of those treated with TGF-β, which exhibited some small protrusions. Organoids of A549 co-cultured with fibroblasts did not display significant differences in morphology (Figure 1).

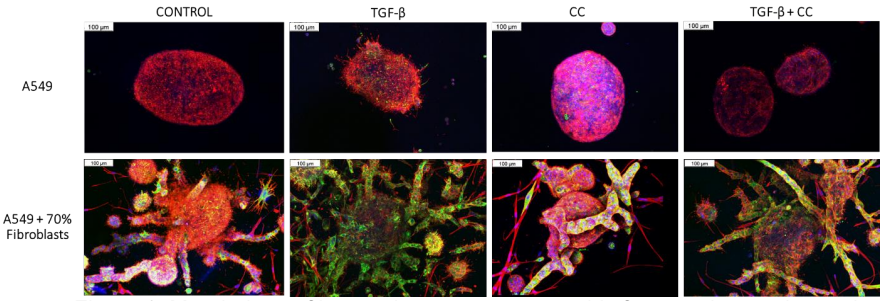

Figure 1. Morphology of human lung cancer organoids from each experimental condition.

Discussion & conclusions: Our findings indicate that A549 spheroids were affected by TGF-β, but organoids were not. This discrepancy may be attributed to the endogenous expression of TGF-βin organoids, which could explain why their morphology remained unaffected. In the combined treatment of cannabinoids and TGF-β, the phenotype induced by TGF-β is reversed in A549 spheroids. Additionally, organoids did not exhibit any morphological changes. Overall, our results provide initial insights into the role of cannabinoid agonists in a 3D *in vitro* model of lung cancer.

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[2] Pacher P., Kogan N.M., Mechoulam R. (2020). Beyond THC and Endocannabinoids. Annu. Rev. Pharmacol. Toxicol. 60, 637–659.

Histology and Histopathology
From Cell Biology to Tissue Engineering

C08 - Histological analysis of the effect of physical exercise on fascicular reorganization of skeletal muscle after injury due to volumetric loss reconstructed with autologous adipose tissue

López-Espejo M.E.[1,2], Moreno-Rey D.[1], Peña-Toledo M.A.[3,4,5], Gil-Belmonte M.J.[1,3,6], Agüera A.[1,3], Jimena I.[1,3,4], Peña-Amaro J.[1,3,4]

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Introduction: In the structural configuration of the muscle extracellular matrix, the perimysium groups a set of muscle fibers delimiting what is known as the muscle fascicle, of great functional importance. In the so-called volumetric loss injury (VML), there is a large loss of muscle mass thatleads to the formation of a cavity that is occupied by fibrous tissue. In reconstruction procedures for this type of injury it would be interesting to assess the degree of recovery of the fascicular structure. Since physical exercise stimulates the regenerative capacity of skeletal muscle, in the present study we analyze the effect of a treadmill exercise regimen on the recovery of fascicular organization in a reconstruction model by autologous adipose tissue transplantation.
Methods: Wistar rats were distributed into 3 experimental groups: normal (N), VML lesion with autologous adipose tissue transplant (AT) and VML lesion with autologous adipose tissue transplant and subjected to a treadmill exercise protocol (AT+EX). A histological, histochemical andhistomorphometric study was carried out at 60 days of evolution, in which the following were determined: number of fascicles/area, cross-sectional area of the fascicles, number of fibers/fascicle and number of fiber types/fascicle. The results were analyzed statistically.
Results: Unlike the N group, in the AT group the newly formed muscle tissue did not present the characteristic fascicular organization as shown by the analyzed parameters, in addition to variability in the size and disorientation of muscle fibers, and loss of the mosaic pattern with an increase in type 2B fibers. The histomorphometric parameters recovered normality in the AT+EX group, beingsignificantly different with the VML group, but not with the N group. Regarding the mosaic pattern,the combination of exercise induced an increase in type 1 and 2A fibers. in addition to reducing thenumber of disoriented muscle fibers.
Discussion and conclusions: The results confirm that the application of an exercise program favorsthe recovery of the normal fascicular structure in a muscle with VML injury reconstructed with autologous adipose tissue. This highlights that physical exercise can be an effective strategy in therecovery of the fascicular organization of skeletal muscle after an injury as severe as VML.

Funding: Supported by grant PS-2020-946 from Consejería de Salud y Familias, Junta de Andalucía, Spain.

C09 - Characterisation of pharmacological model based on lung cancer spheroids/organoids.

Navarro-Esclapés I.[1,2], Monleón-Guinot I.[1,2], Milián L.[1,2] and Mata M[1,2].

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[2] INCLIVA Biomedical Research Institute, Valencia, Spain.

The study of lung cancer and the discovery of new drugs calls for easily reproducible pharmacological models that reliably mimic the complexity of the tumor environment. Three- dimensional models based on *in vitro* generated spheroids/organoids represent a promising pharmacological model. In this study, the hanging drop method was used to generate spheroids/organoids consisting of A549 tumor line cells and normal fibroblasts (NFs) or cancer- associated fibroblasts (CAFs). CAFs were isolated from a biopsy sample from a patient with lung adenocarcinoma and NFs were obtained from peripheral healthy tissue from the same biopsy sample. To mimic the extracellular matrix of the tumor microenvironment, the generated cell droplets were embedded in a type I collagen matrix. Three different types of spheroids/organoids were generated: spheroids consisting of 100% A549 cells, organoids consisting of 30% A549 cellsand 70% NFs and organoids consisting of 30% A549 cells and 70% CAFs [1]. The starting hypothesis of this study was that these spheroids/organoids could be used as a pharmacological model capable of generating a response in the presence of drugs. The spheroids/organoids were treated for 72 hours with different concentrations (100, 10 and 0 µM) of docetaxel, a reference drugused in chemotherapy. After this, cell viability inside the spheroids/organoids was studied by performing an MTS assay. Some spheroids/organoids were used to perform a qPCR to determinechanges in gene expression of marker genes for epithelial-mesenchymal transition, an important event during tumor expansion. Changes in spheroid/organoid morphology were studied byperforming a fluorescence labelling protocol. Phalloidin-rhodamine labelling was used for F-actin localisation (red), immunofluorescence with specific antibody for pankeratin detection (green) and DAPI labelling for cell nucleus detection (blue). Visualization and imaging of the labelled spheroids/organoids was performed using a LEICA TCS-SP8 confocal microscope (Figures 1, 2 and 3). Results indicate that, in addition to the spheroids/organoids exhibiting a dose-dependent response to docetaxel in terms of cell viability and gene expression, the drug also affectsmorphology and cell-cell interactions within the spheroids/organoids. The clear response to the presence of drug would confirm that the proposed model based on spheroids/organoids could be promising as a pharmacological model for the study of lung cancer.

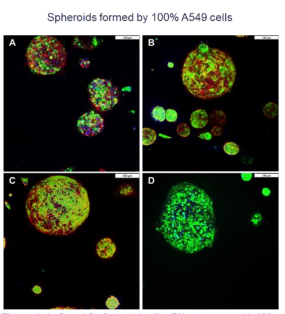

Figure 1. A, B and D: Spheroids after 72h treatment with 100 µM, 10 µM docetaxel, and latex, respectively. C: Spheroids from the drug-free negative control condition.

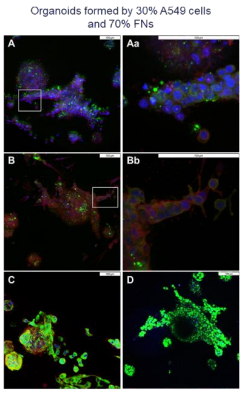

Figure 2. A, C and D: Organoids after 72h treatment with 100 µM, 10 µM docetaxel, and latex, respectively. C: Organoids from the drug-free negative control condition. White squares within the image indicate the beginnings of branching shown in more detail in panels Aa and Bb.

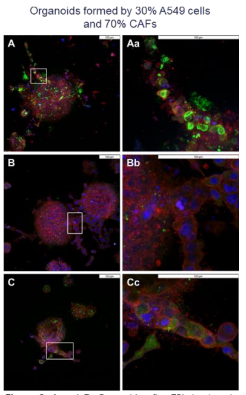

Figure 3. A and B: Organoids after 72h treatment with 100 µM and 10 µM docetaxel, respectively. C: Organoids from the drug-free negative control condition. White squares within the image indicate the beginnings of branching shown in more detail in panels Aa, Bb and Cc.

[1] Monleón-Guinot, I., Milian, L., Martínez-Vallejo, P., Sancho-Tello, M., Llop-Miguel, M., Galbis, J. M., Cremades, A., Carda, C., & Mata, M. (2023). Morphological Characterization of Human Lung Cancer Organoids Cultured in Type I Collagen Hydrogels: A Histological Approach. *International journal of molecularsciences*, 24(12).

C10 - The effects of CS-GPTMS-silica mesoporous hybrid aerogels on survival of human mesenchymal stem cells *in vitro*.

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Introduction: The regeneration of bone defects, resulting from trauma, osteoporosis or tumors, isa problem in our aging society. Stem cells have been used in regenerative medicine with promisingresults due to their capacity for self-renewal, differentiation and regulation. Mesenchymal stem cells(MSCs) play a key role in bone regeneration by differentiating to bone-forming osteoblasts. One ofthe most promising strategies for treating bone defects attempts to mimic the natural bone healingprocess by using MSCs and degradable scaffolds to support cell attachment, migration and proliferation. We previously reported that CS-GPTMS-silica hybrids are biocompatible, bioactive, non-cytotoxic and induce cell adhesion in primary cultures of human osteoblasts [1]. In the presentstudy, we prepared several formulations of hybrid aerogels incorporating different amounts of CS and GPTMS and examined the effect on survival of human mesenchymal stem cells *in vitro*.

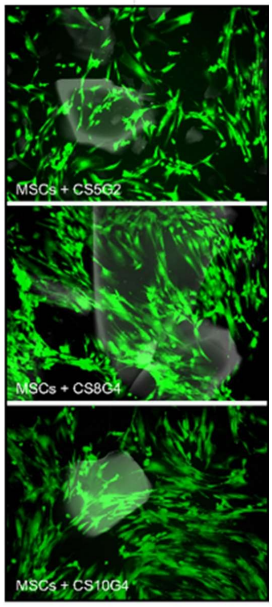

Figure 2: MSCs-aerogels interaction examined with fluorescence microscope (10× objective lens).

Methods:CS-GPTMS-silica hybrid aerogels were prepared by sol gel method. Conversion of hybrids to their respective aerogelswas accomplished by supercritical CO2 drying. The final GPTMS/CS ratio and CS content for each synthesized aerogel are shown in Table 1. MSCs were seeded on the CS-GPTMS-silica hybrids under sterile conditions and after 72 hours, analyzed for cell viability and interaction with materials.

Sample	GPTMS/CS	Final CS Content (wt%)
CS1G0	0	1,7
CS5G2	2	3,1
CS8G4	2	3,1
CS10G4	4	9,7

Table 1: Sample identification, GPTMS/CS ratio and final content of CS based on Elemental Analysis

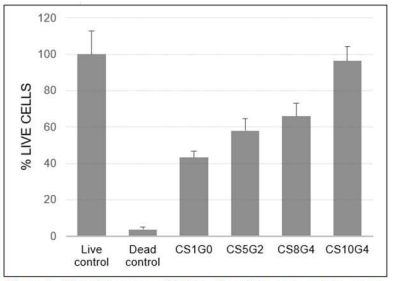

Figure 1: Citotoxicity assay of MSCs after 72 hours in culture, grown in the presence of aerogels.

Results: Cell survival assays with the different materials revealed a differential effect on MSCs. MSCscultured with CS10G4 had a survival of 96,225 %, CS8G4 of 65,840 %, CS5G2 of 57,952 % and CS1G0 of 43,132 % (Figure 1). Furthermore, usingfluorescence microscopy we verified that the cells interact with all the materials studied (Figure 2). Discussion & conclusions: These results demonstrate that both higher GPTMS/CS ratio and higher percentage of CS positivelyinfluence the survival of MSCs. We observed that the presence of GPTMS/CS has a positive effect on the interaction of the surviving MSCs in all the CS-GPTMS-silica hybrid aerogels studied. Therefore,we can conclude that the CS-GPTMS-silica hybrid aerogels togetherwith MSCs, studied in this work, are a promising strategy as a combined treatment for bone diseases.

[1] Reyes-Peces M. et al (2020). Chitosan-GPTMS-Silica Hybrid Mesoporous Aerogels for Bone Tissue Engineering. Polymers (Basel). 17;12(11):2723. doi: 10.3390/polym12112723.

Support: University of Cadiz Plan Propio (Grant for PR2023-017) and Junta de Andalucia (Spain): CTS-253 PAIDI (Tissue Engineering Group) and TEP115 Group.

Oral Presentations

Sessions of Wednesday 11th (16:30h -17:30h)

Oral 3 - General Histology I

Moderators:
Alberto Enrique D'Ottavio
Alejandro Escamilla Sánchez

Oral 4 - Tissue Engineering II

Moderators:
Alejandro Romero Rameta
Óscar Darío García García

C11 - A histologic and morphometric study of non-photodependent human skin aging.

Marta Arnal-Forné[1], César Ríos-Navarro[1,2,+], Tamara Molina-García[2], María Ortega[1], Víctor Marcos-Garcés[2,3], Pilar Molina[4], Antonio Ferrández-Izquierdo[1,2,5], Pilar Sepulveda[1,6,7], Vicente Bodí[2,3,6,8], Amparo Ruiz-Saurí[1,2,+]

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[2]INCLIVA Biomedical Research Institute. Valencia, Spain.
[3]Cardiology Department. Hospital Clínico Universitario. Valencia, Spain.
[4]Department of Pathology, Instituto de Medicina Legal y Ciencias Forenses, Valencia, Spain.
[5]Anatomic Pathology Department. Hospital Clínico Universitario. Valencia, Spain.
[6]Centro de Investigación Biomédica en Red (CIBER)-CV. Madrid, Spain.
[7]Regenerative Medicine and Heart Transplantation Unit, Instituto de Investigación Sanitaria La Fe, Valencia, Spain.
[8]Department of Medicine, University of Valencia. Valencia, Spain.

Skin constitutes the main barrier against external environment [1], but it also participates in humanrelationships, being one of the most important beauty determinants [2] and a major pharmaceuticalclaim. Since skin aging is an important factor in medical and social spheres, this basic science research (approved by the local Research Ethics Committee) aims to characterize microscopic changes in human skin composition due to intrinsic aging (the one not influenced by external factors) [3], via histological analysis of periumbilical skin samples, a photoprotected body region. The samples from 25 autopsies (Valencia Clinical Hospital), were classified into age groups: children (0-12), youth (13-25), middle-aged adults (26-54), elderly (>55 years). Afterwards, different histological (Hematoxylin-Eosin, Masson's Trichrome, Orcein, Toluidine, Alcian blue, Feulgen), and immunohistochemical techniques (CK20, CD1a, Ki67, CD31) were performed. After being photographed with Leica DM3000 optical microscope, images were morphometrically analyzed using Image ProPlus 7.0 for further statistical analysis with GraphPad 9.0. Results regarding epidermis showed a decrease in its thickness (p <0.05), interdigitation andmitotic indexes (p <0.01), while melanocytes (p <0.05) were raised. In papillary dermis, mast cells (p <0.05) and glycosaminoglycans (p <0.001) were expanded, whereas in reticular dermis a reduction of glycosaminoglycans (p <0.01) and elastic fibers (p <0.05)was perceived. Moreover, total cellularity (p <0.05) and vascularization (p <0.01) of both dermis were diminished with aging. This morphometric analysis of photoprotected areas reveals that intrinsic aging significantly influences human skin composition. This research establishes the basis of intrinsic aging consequences and opens the door to further investigations on molecular matter regarding the grounds of chronological skin aging [4].

[1] Csekes, E., & Račková, L. (2021). Skin aging, cellular senescence and natural polyphenols. International journal of molecular sciences, 22(23), 12641.

[2] Sakano, Y., Wada, A., Ikeda, H., Saheki, Y., Tagai, K., & Ando, H. (2021). Human brain activity reflectingfacial attractiveness from skin reflection. Scientific Reports, 11(1), 3412.

[3] Fisher, G. J., Kang, S., Varani, J., Bata-Csorgo, Z., Wan, Y., Datta, S., & Voorhees, J. J. (2002). Mechanisms of photoaging and chronological skin aging. Archives of dermatology, 138(11), 1462-1470.

[4] Zhang, S., & Duan, E. (2018). Fighting against Skin Aging: The Way from Bench to Bedside. Cell Transplantation, 27(5), 729–738. https://doi.org/10.1177/0963689717725755

C12 - Biomarkers to estimate vulnerability from sea anemone *Bunodosoma cavernatum*

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Introduction: The global warming has modified the weather patterns, causing damages and alterations in marine organisms. A group of marine organisms affected, are the sea anemones, organisms with ecologic, economic, touristic and biotechnology importance. These organisms employ biological strategies to survive, like the tissue regeneration and the synthesis of proteins, shaping their adaptative capacity. The adaptative capacity represents a parameter that can be associated with exposition and sensibility to evaluate vulnerability (1). The aim of this research is to evaluate the adaptative capacity in the anemone *B. cavernatum* inducing a wound and evaluatingthe responses through tissular and proteinic biomarkers, besides a proteinic analysis of organismsexposed to a thermal stress to identify the behavior to climate change.

Methods: Sea anemones were collected on the Gulf of Mexico, Tecolutla, Veracruz. They were placed in a quarantine aquarium for 2 weeks, subsequently, the physicochemical conditions were adjusted for its maintenance. The sedation of the organisms was carried out with a temperature gradient, which consisted of partial water changes (from 24 ºC to 4 ºC). To evaluate the regenerative capacity, a wound was made in the region of the column. Subsequently at 8, 24, 48, 72 y 648 hours (h) we obtained a biopsy for thehistopathological and proteic analysis. For the temperature change analysis, oneexperimental group was submitted at a variable temperature regime (26-31 °C) and another group was submitted at a constant temperature regime (27 °C) for 1 month, the organisms were subsequently subjected to thermal stress (32°C) for 2 weeks and its proteome was analyzed by mass spectrometry techniques.

Results: The tissular and proteinic responses were used as indicators to evaluate the adaptative capacity within the model of vulnerability. The tissular and proteic characterization allowed to describe the metabolic basal state, enabling to identify main tissue structures such as the Muscular Epithelium, the Mesoglea and the Mesenteries, inaddition to other cells, such as Amebocytes. In the bioassaybiopsies, cell activation was observed at 8 h, structural changes during the different experimental times (Fig. 1), and muscle regeneration at 72 h (Fig. 2). At 648 hours the tissue structure was reconstituted. The proteomic analysis showed the presence of proteins that intervene in thebiological responses associated to the tissular responses like the regeneration and the thermal stress. Biological responses were quantified to assign a numerical value in the vulnerability model.

Discussion & Conclusions: The regeneration is a biological response that can be related with the adaptative capacity of the sea anemones. The integration and quantification of tissue and protein analysis, allow us to estimate the vulnerability of this type of organisms to climate change.

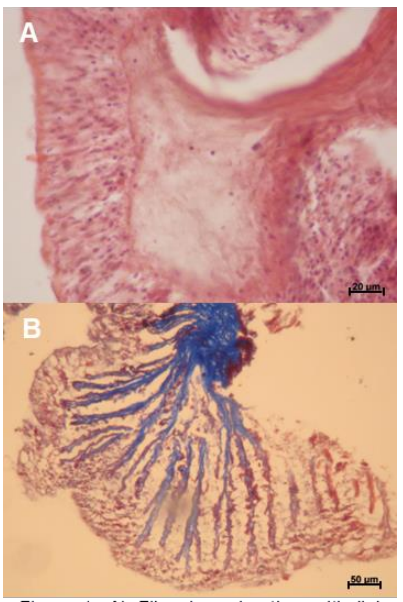

Figure 1. A) Fibrosis under the epithelial tissue showed at 24 hours. B) Mesenteries with positive stain to collagen inside the structures at 72 hours.

[1] Seaborn, T., Griffith, D., Kliskey, A., and Caudill, C. C. (2021). Building a bridge between adaptive capacity and adaptive potential to understand responses to environmental change. *Global Change Biology*, *27*(12), 2656-2668.

C14 - A spinal cord injury regeneration model based on informational structures derived from Lotka-Volterra dynamics

Esteban FJ.[1], Ruiz-Amezcua P.[1,2], Langa JA.[3], Soler F.[4], Caraballo T.[3], Nieto-Díaz M.[2]

[1]Unidad de Biología de Sistemas, Departamento de Biología Experimental, Universidad de Jaén, Spain.
[2]Grupo de Neuroprotección Molecular, Unidad de Neurología Experimental, Hospital Nacional de Parapléjicos (Servicio de Salud de Castilla-La Mancha) Toledo, Spain.
[3]Departamento de Ecuaciones Diferenciales y Análisis Numérico, Universidad de Sevilla, Campus Reina Mercedes, 41012 Sevilla, Spain.
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Introduction: We introduce a spinal cord injury regeneration model, based on the informational structures derived from Lotka-Volterra dynamics [1-3], to explore and quantify the complex interactions essential for tissue recovery post-injury. The model is related to our recent work in which the analysis of informational structures allows for characterizing different states of consciousness [3]. With this model, we address the complex cellular interactions conditioning axonal regeneration after spinal cord injury, aiming to demonstrate the utility of informational structures in understanding the dynamics of the nervous tissue.

Methods: The model is based on the characterization of informational structures arising from Lotka-Volterra dynamics [1-3]. Following spinal cord injury, we specifically simulate the axonal rupture, activation of two types of astrocytes: A1, inhibiting axonal regeneration, and A2 as facilitators. We also include the disruption of oligodendrocytes leading to myelin release (as an inhibitor of axonal growth and regeneration) and the parallel proliferation of oligodendrocytes to remyelinate demyelinated axons. Neurons do not strongly activate regenerative states on their ownand their growth dynamics are influenced by the overall cellular environment. Using the frameworkof informational structures, the dynamics and stability of the system under perturbations are evaluated.

Results: The stability of graphs associated with the informational structures detected from Lotka-Volterra dynamical systems (Figure 1) shows that the interactions mediated by populations of astrocytes, especially type A2, along with the modulation of the inhibitory effects of myelinand oligodendrocytes, induce significant improvements in the regenerative process. In fact, the viability of A2 astrocytes as critical promoters in axonal regeneration is presented, together to the influence of the inhibitory signalsfrom oligodendrocytes and its proliferative state.

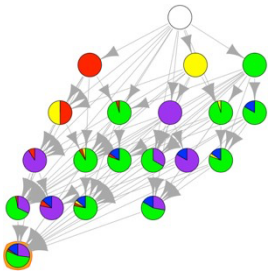

Figure 1. Informational structure. Red:A1; green: A2; blue: neurons; yellow: myelin; purple:

Discussion and Conclusions: The proposed model, integrating the informational structures based on Lotka-Volterra dynamics, provides a new perspective on the axonal regeneration following spinal cord injuries. This approach allows us the quantification of specific interactions between the various cellular types involved, primarily A2 astrocytes and oligodendrocytes, two critical components in the tissue connectivity matrix, and their impact on axonal regeneration post-injury. The predominant activity of both A2 astrocytes and oligodendrocytes at the injury site have been identified as crucialfor maintaining neuronal presence. In conclusion, the model is presented as a robust quantitative tool for designing targeted therapeutic strategies that enhance the activity of A2 astrocytes and, in turn, suppress the negative effects of oligodendrocytes, which could constitute a significantadvance in regenerative medicine and tissue engineering.

[1] Esteban F.J., Galadí J.A., Langa J.A., Portillo J.R., Soler-Toscano F. (2018). Informational structures: A dynamical system approach for integrated information. PLoS Comput. Biol. 4(9), e1006154.

[2] Almaraz P., Kalita P., Langa J.A., Soler-Toscano F. (2024). Structural stability of invasion graphs for Lotka-Volterra systems. J. Math. Biol. 88(6), 64.

[3] Soler-Toscano F., Galadí J.A., Escrichs A., Sanz Perl Y., López-González A., Sitt J.D., Annen J., Gosseries O., Thibaut A., Panda R., Esteban F.J., Laureys S., Kringelbach M.L., Langa J.A., Deco G. (2022).What lies underneath: Precise classification of brain states using time-dependent topological structure of dynamics. PLoS Comput. Biol. 18(9), e1010412.

C15 - Improvement of human dental pulp stem cells neurodifferentiation methods to obtain functional neuron-like cells.

Pardo-Rodríguez B.[1], Baraibar Andrés M.[1,3,5], Manero-Roig I.[1,4], Luzuriaga J.[1], Salvador J.[1], Basanta-Torres R.[1], Polo Y.[2], Unda F.[1], Mato S.[1,3,5,] Ibarretxe G. [1*†] and Pineda J.R.[1,3*†]

1 University of the Basque Country (UPV/EHU), Leioa, Spain
2 Polimerbio SL, Donostia-San Sebastian, Spain
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*Corresponding authors. †These authors contributed equally to this work.

The lack of effective treatment options combined with the rising incidence and prevalence of brain lesions and neurodegenerative diseases has brought attention into stem cell based neuroregenerative therapies, which may be able to repair damaged neural tissue through paracrineeffects or cell replacement. In this context, human dental pulp stem cells (hDPSCs) have emergedas a promising alternative due to their unique features including their neural crest origin, their abilityto release neuroprotective and immunomodulatory factors and to express mature neuronal and glial markers. However, it is still necessary to get a better understanding of these cells' true capacityto give rise to functional neurons, which could fire action potentials and integrate into a complex synaptic network. Thus, in this work we have characterized the differentiation of hDPSCs using twodifferent protocols previously used to neurodifferentiate neural stem cells focusing on both neuronalprotein expression, cell morphology and their electrophysiological properties.

hDPSCs were extracted from third molars of healthy donors and were expanded *in vitro* either with or without the presence of serum. Then, parallel cohorts of cultures were split and switched toneural differentiation either using NeuroCult Differentiation media or this media improved with retinoic acid (RA 3mg/ml) and 40 mM KCl pulses. Both RA and KCl have been previously demonstrated to commit neural stem cells to a GABAergic phenotype after differentiation. Differentglial, neuronal and synaptic marker expression was measured by immunocytochemistry and quantitative Real-Time PCR. Cell and nuclear morphology were characterized using 3D-Sholl analysis. Neurodifferentiated hDPSC functionality was measured by electrophysiological recordings. After inducing hDPSC neurodifferentiation, we observed a significant reduction in stem cell markers as well as an expression of mature neuronal and glial markers, some of which were expressed differently in cells that had been previously grown in serum-containing media with respect to serum-free media. Besides, 3D-Sholl analysis showed smaller cell bodies with long, thinand ramifying processes in cells cultured without serum. Despite this, all differentiated hDPSCs expressed the building blocks required to form GABAergic synapses as well as glutamatergic ones,hDPSCs treated with KCl and RA showed a higher expression of some neurotransmitter receptorsand voltage dependent channels which made them able to generate voltage-dependent Na^+ and K^+ currents as well as action potentials.

Our results highlight the importance of choosing an appropriate protocol to neurodifferentiate hDPSCs into functional neuron-like cells. The addition of serum, even transiently, elicits long-lasting changes in cellular morphology that are not reversed after the neurodifferentiation protocol. The *invitro* expression of synaptic markers, ionotropic neurotransmitter receptors and functional voltage dependent Na^+ and K^+ channels suggest the possibility of their successful integration into the neuronal synaptic network *in vivo*.

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C16 - Enzymatically Decellularized Human Adipose Tissue Solid Foams enhance adipogenic *in vitro* and *in vivo* differentiation of human Dental Pulp Stem Cells.

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Fat exerts energetic and hormonal control in the body. Although its removal methods are widely known, fat implants are also used for aesthetic reasons, breast reconstruction after lumpectomy orto correct contour deformities. However, in recent years, many avenues of research have been opened to try to obtain brown fat adipocytes. Brown fat is common in the adipose panniculus of infants (layer of adipose tissue under the skin) and is intended to maintain their body temperature.The generation of brown fat can be promoted by exposure to cold, through physical exercise or through the ingestion of certain foods and has been proposed, in the context of metabolic diseases,could be an effective tool for maintaining good health. Recent works suggest that the transformationof white fat into brown fat may be beneficial for the prevention of prostate cancer, or the possibilityof burning nutrients and reducing the excess of calories to produce heat could have a protective effect against obesity. Furthermore, by draining the excess glucose from the blood to be oxidized becoming a current research therapy against diabetes. One of the major drawbacks and limitations is that this “heat-generating” form of fat is known to disappear with age, being reduced to small tissue niches in the vicinity of the kidneys, thymus or at the base of the neck. For all these reasons, the possibility of generating and transplanting scaffolds with the "correct" fat could open up new therapeutic possibilities for all these types of alterations.In the present work, we propose to use the adipogenic differentiation capacity of human dentalpulp stem cells (hDPSCs) combined with enzymatically human decellularized adipose tissue (hDAT) biologic scaffolds. hDAT was processed as solid foams for the growth, differentiation and subsequent implantation of hDPSC-derived fat to test the feasibility of the system as a preliminary option for a potential therapeutic approach. We designed, characterized and validated extracellularmatrix (ECM)-based materials of pure hDAT, bovine collagen-I (bCOL-I) (control) or a combinationof both in a 3:1 proportion (3hDAT:1bCOL-I), to reduce costs and recreate a microenvironment compatible with stem cell survival and differentiation. hDAT was obtained by a two-enzymatic stepdecellularization protocol and further processed by freeze-drying to obtain 3D solid foams. We found that all the assayed solid foams supported hDPSC viability and proliferation. Incubation of hDPSCs with adipogenic medium in pure hDAT-based solid foams increased the expression of mature adipocyte LPL and c/EBP gene markers as determined by RT-qPCR, with respect to bCOL-I solid foams. Moreover, hDPSC capability to differentiate towards adipocytes was assessed by PPAR-γ immunostaining and Oil-red lipid droplet staining. Our mixed composition of 3hDAT:1bCOL-I foams was optimal to support adipogenesis in 3D-hDPSC cultures. Next, to test the viability and integration of the devices in a more real scenario, we subcutaneously implanted 3hDAT:1bCOL-I solid foams in immunosuppressed nude mice. One-month post-implant we sacrificed the animals and performed histological characterization. H/E analysis showed a large structure of collagen and the presence of vascularized tissue integrating the foams. In addition, in agreement with the data observed *in vitro,* 3hDAT:1bCOL-I foams loaded with hDPSCs displayeda major vascularized cellular presence of adipocytes. Interestingly, adipocytes generated after *in vivo* transplantation corresponded to a multilocular brown fat histologic phenotype.In conclusion, the generation of 3hDAT:1bCOL-I solid foams could support both *in vitro* and *in vivo* adipogenesis facilitating the widespread use of autologous stem cells therapy and biomaterials for personalized or aesthetic medicine.

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C17 - Proteomic analysis of decellularized extracellular matrix scaffolds derived from porcine reproductive tissues.

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Introduction: The extracellular matrix (ECM), composed of a basal membrane and an interstitial matrix, not only provides physical support to cells but also harbors proteins and growth factors thatstimulate cell migration, proliferation, and differentiation. Decellularization, a procedure that isolatesthe ECM from organs or tissues, effectively removes all cells while preserving the essential components of the ECM. The isolated matrix, known as a dECM scaffold, has diverse applicationsin tissue engineering and regenerative medicine. In the field of reproduction, these scaffolds are used to treat pathologies that affect reproductive function or to enhance assisted reproductive technologies (ART). This study aimed to apply a decellularization protocol to porcine oviducts anduterine sections and subsequently evaluate, through proteomic analysis, the efficacy of the protocol in preserving key extracellular matrix proteins. Methods: The decellularization procedure was performed on oviducts and uterine sections from prepubertal female pigs (Large White x Landrace) using a modified protocol [1]. Initially, the organs underwent a freeze-thaw cycle before starting the decellularization protocol. The decellularization protocol consisted of two identical 24-hour cycles with alternating exposures to sodium dodecyl sulfate (SDS) and Triton X-100, followed by enzymatic treatment with DNase and final washes withPBS for 48 hours. Upon completion of the protocol, samples from both organs were collected for proteomic study. Samples from non-decellularized organs were also taken as controls. Protein extraction was performed using a lysis buffer (50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 0.25% SDS,HALT protease inhibitor cocktail without EDTA). The proteins were then quantified using the Bradford assay and visualized with SDS-PAGE. Subsequently, the samples underwent trypsin digestion and were analyzed by HPLC-MS/MS. The data obtained were compared against the porcine protein database from Uniprot. Finally, bioinformatic and statistical analyses were conducted, including tools such as VennDiagram and FactoMineR to identify unique proteins and perform principal component analysis (PCA). Proteins showing significant differences (FDR ≤ 0.05) underwent further enrichment analysis in DAVID for the study of gene ontology (GO) terms. Results: Venn diagrams revealed 241 proteins common between both control and decellularized groups in oviducts and 138 in uterine sections. The PCA clearly segregated the control and decellularized groups, demonstrating distinct differences in their proteomic profiles. Samples withineach group exhibited a consistent proteomic pattern, with the decellularized group showing greateruniformity. The GO analysis identified a notable presence of ECM-related components such as "extracellular space" and "extracellular region" in both the oviducts and decellularized uterine sections. The biological processes identified in the decellularized samples were related to protein organization and folding. The most relevant molecular processes were those related to protein and receptor binding. The study also highlighted the importance of collagen and key macromolecules such as laminin, filamin, dermatopontin, and fibronectin, which are essential for the matrix structure and cellular functions. Specifically, dermatopontin and fibronectin play crucial roles in cellular signaling and could significantly enhance blastocyst hatching in reproductive applications. Conclusion: The proteomic analysis conducted on uterine and oviductal scaffolds revealed numerous proteins common to both decellularized tissue and control, confirming that the decellularization protocol effectively preserves tissue proteins.

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Acknowledgments: Ministerio de ciencia e innovación (PID2019-106380RB-I00 MCIN/ AEI /10.13039/501100011033) y (PID2021-12309NB-C21 MCIN/AEI/10.13039/ 501100011033). Contrato predoctoral Plan de Fomento de la Investigación de la Universidad de Murcia para 2022 (R-496/2022).

C18 - Optimization of the biological and biomechanical properties of hydrogels scaffolds used in tissue engineering

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Introduction: The recent development of tissue engineering allows the treatment of patients with severe damage of the skin, cornea and nerve [1]. Although current technology made possible the generation of bioartificial tissue substitutes, the biological and biomechanical properties of these novel medicinal products should still be enhanced. One of the alternatives is the use of crosslinkingagents, such as genipin (GP), which previously demonstrated to be capable of increasing the mechanical properties of several types of hydrogels [2]. This study aims to evaluate the biological properties of decellularized membranes crosslinked with GP for use in tissue engineering.
Methods: Decellularized pericardium membranes were treated with increasing concentrations of GP (0.01%, 0.1%, and 1%) for 24 hours. Ex vivo evaluation was then performed through indirect coculture with macrophages for 24 hours. Viability and metabolic activity assays were performed, and macrophage phenotypic profile changes were assessed. Subsequently, the material demonstrating the most promising ex vivo outcomes was chosen for in vivo analysis by subcutaneous grafting in four different anatomical regions of the dorsal area of BALB/c laboratory mice, along with the control group. Mice were then monitored for 10- and 30-days post-implantation,and in vivo biocompatibility was evaluated through hemogram and histological assays.
Results: Results in indirect cultures demonstrated that crosslinked biomaterials did not exhibit anycytotoxic effects on macrophages, as determined by live/dead assays. Metabolic activity analysis showed an increase of macrophages in contact with GP-treated membranes, reflecting high rates of cell proliferation. The polarization assays showed a pro-regenerative phenotype macrophage polarization in GP-treated membranes, as evidenced by the CD86/CD163 ratio. Then, in vivo analysis demonstrated no apparent rejection and, during the extraction, seamless integration of thebiomaterial into the adjacent tissue was observed. Finally, hemogram and histological studies confirmed no side effects and an optimal integration of GP-treated membranes.
Discussion & conclusions: Our study demonstrated that GP-crosslinked decellularized membranes showed enhanced biological properties. Ex vivo assessments revealed increased metabolic activity and a pro-regenerative macrophage phenotype, while in vivo analysis confirmed the proper biointegration of these biomaterials. These findings highlight the potential of GP crosslinking to improve the suitability for skin, cornea and nerve tissue engineering applications.

Acknowledgements: This work was supported by C-CTS-032-UGR23, Plan Propio de Investigación y Transferencia de la Universidad de Granada 2023, Consejería de Universidad, Investigación e Innovación, Junta de Andalucía. Funded by the European Union, Fondo Europeo de Desarrollo Regional ERDF-FEDER, Programa Operativo FEDER Andalucía 2021-2027, and grant PE-0395- 2019 from Consejería de Salud y Consumo, Junta de Andalucía, Spain. Supported by grants FIS PI23/00335, FIS PI23/00337 and FIS PI22/00059, funded by Instituto de Salud Carlos III (ISCIII), Ministry of Science, Innovation and Universities (Plan Estatal de Investigación Científica, Técnica y de Innovación 2021-2023, and Plan de Recuperación, Transformación y Resiliencia), and co- funded by the European Union, Fondo Europeo de Desarrollo Regional ERDF-FEDER.
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Oral Presentations

Sessions of Thursday 12th (10:30h -12:30h)

Oral 5 - Tissue Engineering III

Moderators:
María José Izquierdo Rico
Natalio García Honduvilla

Oral 6 - Histopathology

Moderators:
José Antonio Gómez Sánchez
José María Fernández Santos

C19 - An in vitro characterization and in vivo bio-compatibility assessment of printable biomaterials for neural engineering applications

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Introduction: Nervous system traumatic injuries are the main cause of morbidity, disability and even mortality among young adults in industrialized nations [1]. The neural tissue complex structure and limited intrinsic regenerative capacity have meant that standard treatments for these injuries are often ineffective and come with numerous disadvantages [2]. In the search for innovative alternatives, 3D printing technology has made significant advances in neural tissue engineering field, enabling the generation of biomimetic neural substitutes [3]. In this context, the aim of this work was to screen biomaterials for generating 3D printed scaffolds for use in neural engineering.
Methods: Here four thermoplastics biomaterials [polycaprolactone (PCL), polylactic acid (PLA), Filaflex (FF) (assessed here for the first time for biomedical purposes) and Flexdym (FD)] and a gelatin methacrylate (GelMA) hydrogel were selected. They were subjected to printability tests with the REG4LIFE bioprinter and to mechanical characterization by uniaxial tensile testing to fracture (Instron Model 5943). Moreover, *in vitro* cell-biomaterial interaction analyses were carried out by seeding neural cells (SH-SY5Y) within the scaffolds and the cell viability index and metabolic activity were determine after 72 hours and 7 days *in vitro*. Finally, for the *in* vivo biocompatibility assessment, scaffolds were subcutaneously implanted in Lewis rats, and after 10 days, collected samples were histologically analyzed.
Results and Discussion: Thermoplastics biomaterials showed superior printing results in terms of resolution and shape-fidelity, especially PLA, FF and FD. In relation to the mechanical characterization, FD and GelMA revealed great viscoelastic properties and, additionally, FD exhibited the highest elasticity. According to the *in vitro* assay, after 72 hours of cell culture, it appears to be a similar number and proportion of viable cells in all groups, however, GelMA demonstrated the greatest cell viability index after 7 days of cell culture, as expected due to its natural properties. In the *in vivo* assay, it was observed the presence of a well-defined pseudocapsule, composed by an inner cellular layer and an external fibrotic layer, around all grafted scaffolds with slight differences. CD45 immunohistochemistry revealed that most cells observed around scaffolds were of the immunological lineage, and no signs of polymorphonuclear infiltration were observed.
Conclusions: This study evaluated various biomaterials for neural engineering applications, finding promising results with desirable mechanical and biological properties. Thermoplastics biomaterials resulted good candidate for 3D printing technology with better biomechanical properties and acceptable biocompatibility. Future studies will be designed to determine the usefulness of these biomaterials in the generation of novel engineered substitutes for peripheral or central nervous system repair studies.
Fundings: This research was financed by grants PI23/00337 and FIS PI20/00318, funded by Instituto de Salud Carlos III (ISCIII), Ministry of Science, Innovation and Universities (Plan Estatal 2021-2023, and Plan de Recuperación, Transformación y Resiliencia). Co-funded by the European Union, Fondo Europeo de Desarrollo Regional ERDF-FEDER. Funded by grant CPP2021-009070 by “Proyectos de colaboración público-privada”, Plan de Investigación Científica, Técnica y de innovación 2021-2023, Ministerio de Ciencia e Innovación, Unión Europea, Agencia Estatal de Investigación, España.

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C20 - Extracellular matrix from porcine decellularized adipose tissue: A versatile bioscaffold for three-dimensional hDPSC culture and tissue engineering

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Introduction: The combination of human stem cells with extracellular matrix (ECM) derived biologic scaffolds is a promising approach for the development of tissue engineering and regenerative medicine therapies. These *in vitro* generated three-dimensional (3D) humanized tissues are also of great value to the pharmaceutical industry because they faithfully reproduce the cell-ECM interactions that take place in live organisms, all the while reducing the amount of animals used for experimental purposes. Human Dental Pulp Stem Cells (hDPSCs) are a very powerful and accessible stem cell source to generate diverse types of mature cell lineages. When grown under precise combinations of factors, hDPSCs can give rise to different types of specialized cells, including bone, adipose, vascular and neural cells [1]. The synergy between the research groups of TECNALIA and UPV/EHU led to the generation of 3D culture systems of hDPSCs on ECM from porcine decellularized adipose tissue (pDAT) [2]. This pDAT can be further processed in different formats such as solid foams and *in situ* polymerizable hydrogels with customized geometry, porosity and density.
Methods: 3D cultures (1 to 4 weeks) of hDPSCs were made using pDAT bioscaffolds formulated as solid foams at various concentrations between 0.25% (w/v) and 1% (w/v) comparing them with commercial Collagen type I, and hydrogels at various concentrations between 0.75% (w/v) and 3% (w/v), comparing them with commercial Matrigel™. Different protocols of osteogenic and vasculogenic differentiation were applied to trigger the generation of both tissues of interest. The generation of bone tissue was assessed by Alkaline Phosphatase (ALP) activity, bone matrix deposition and mineralization (Alizarin Red staining, Transmission Electron Microscopy) and the expression of mature bone markers like Osteocalcin and Osterix/SP7 by immunostaining and RT- PCR. The generation of vascular tissue was assessed by immunolabeling of capillary like structures expressing Nestin, CD146, von Wildebrand Factor and CD31. Cell viability was assessed by calcein/propidium iodide staining. Statistical analysis was performed by IBM SPSS Statistics v28.1.
Results: All pDAT derived biologic scaffolds showed an optimal biocompatibility with hDPSCs, with cell viability rates approaching 100% at 1 week in culture. Solid foams were particularly effective to bolster the generation of calcified bone tissue after 4 weeks in culture, with higher mineralizing rates compared to Collagen type I solid foams, and correlating with higher porosity of the bioscaffold. PDAT hydrogels showed superior cell encapsulating properties compared to Matrigel™. Endothelial vWF+/CD31+ cells and Nestin+/CD146+ pericytes were detected in both pDAT solid foams and hydrogels at 2 weeks in culture, showing the potential of these systems to undergo vascularization.
Discussion & conclusions: PDAT is an extremely versatile biomaterial showing excellent processing properties and compatibility with differentiation protocols of hDPSCs for *in vitro* tissue modeling. The use of xenogenic ECMs has been amply tested by the biopharmaceutical industry to promote tissue healing and regeneration, and translated into a plethora of products for clinical use. Given the high availability of porcine adipose tissue, this system could be used to generate tailored tissue engineering constructs for drug screening and regenerative medicine.

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C21 - Generation of bioengineered substitutes of the human sclero- corneal limbus using decellularization protocols

Ávila-Fernández P.[1,2], Chato-Astrain J.[1,2], García-García O.D.[1,2], Alaminos M.[1,2], Etayo-Escanilla M.[1,2], Sánchez-Porras D.[1,2], Garzón I.[1,2], González-Gallardo C.[1,2,3], Campos F.[1,2]

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Introduction: Human epithelial limbal stem cells (hELC) residing at the sclero-corneal limbus are vulnerable to damage, resulting in limbal deficiency and blindness. Transplantation of hELC is the gold-standard therapeutic approach for regenerating the damaged corneal surface in patients with limbal stem cell deficiency, although long-term success rates are limited [1]. Several limbal substitutes have been developed by tissue engineering to host functional hELC. However, the complexity and microarchitecture of the limbus region makes it very difficult to reproduce in the laboratory. In this study, we generated sclero-corneal limbal substitutes that could have future therapeutic potential.
Methods: Human limbi were decellularized using two different protocols and analyzed to evaluate DNA removal, extracellular matrix preservation and biocompatibility. Then, limbi were recellularized using cultured human corneal epithelial cells (hCECs). Substitutes were maintained 7 days in a cell incubator using standard culture conditions at 37ºC with 5% CO_2, and histological and immunohistochemical analyses were performed to evaluate cell adhesion and the expression of several limbal markers.
Results: Both protocols achieved effective decellularization results, optimal for the recellularization process. Histological analysis of the recellularized substitutes showed that both limbal substitutes were able to sustain cell adhesion and proliferation of cells used to recellularize these scaffolds. Recellularized limbi showed an epithelial layer consisting of a single cell stratum attached to the decellularized scaffold. Immunohistochemical analysis of limbal markers revealed positive expression of cytokeratin AE1/AE3 and crystallin lambda (CRY-λ) in both limbal substitutes.
Discussion & conclusions: In this study, we developed limbal substitutes using two decellularization techniques able to preserve the structural complexity of the human limbus. These substitutes exhibited promising results regarding cell adhesion and proliferation. Furthermore, the expression of epithelial markers and corneal-specific markers highlighted the ability of decellularized scaffolds to support cell differentiation. These results suggested that the limbal substitutes generated in this work share important similarities with the native limbus, and support their future clinical application in patients with limbal deficiency.
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C22 - Development of new hyaline cartilage substitutes based on the combination of different three-dimensional culture strategies

Sánchez-Porras D.[1,2], Campos F. [1,2], Ortiz-Arrabal O[1,2], Ávila-Fernández P[1,2], Etayo- Escanilla M.[1,2], Chato-Astrain J.[1,2] García-García O.D.[1,2], Alaminos M[1,2], Carriel V.[1,2]

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Introduction: There are several pathologies that can affect hyaline cartilage structures, among which degenerative diseases, such as osteoarthritis, or traumatic diseases, stand out due to their high prevalence in society [1]. These pathologies usually have a difficult prognosis due to the special intrinsic characteristics of hyaline tissue, and there is still no effective long-term strategy for the treatment of these lesions. In this study, we evaluated the possibility of generating new cartilage substitutes by combining microtissues [2], highly biomimetic three-dimensional structures derived from hyaline chondrocytes with natural biomaterials of demonstrated regenerative efficacy in other tissues, such as fibrin and agarose [3].
Methods: Chondrocyte-based microtissues were generated for 7 days using chondrogenic culture conditions by using agarose-microchip cell culture technique [2]. Microtissues and dispersed chondrocytes were encapsulated in fibrin-agarose hydrogels (FA-MT and FA-C, respectively) and characterized in terms of viability and metabolic activity during 28 days of *ex vivo* development. Moreover, histological analyses were conducted to determine the behavior of the encapsuled MT in the biomaterial and dispersed control chondrocytes.
Results: Hyaline cartilage substitutes reveal high viability index during the whole period analyzed, demonstrating a high biocompatibility of the system applied to chondrocyte microtissues using this biomaterial. In addition, increased synthesis of molecules that constitute the main components of the cartilage-specific extracellular matrix, such as aggrecan proteoglycan and type II collagen, and of the pericellular matrix components, such as type IV collagen and perlecan proteoglycan, was observed when microtissues were applied to generate the substitutes compared to dispersed chondrocytes.
Discussion & conclusions: Our results demonstrate the possibility of generating artificial hyaline substitutes by combining fibrin-agarose hydrogels with human hyaline chondrocytes, being the synthesis of specific extracellular matrix clearly enhanced when the cellular component has been previously cultured as microtissues. However, the regenerative potential of the generated substitutes should be evaluated in future experiments on a cartilage injury model.
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C23 - Comparative evaluation of nerve-repair approaches for long-gap defects in rat models

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Introduction: Starting with the initial development of a peripheral nerve substitute using a silicone elastomer tube, numerous conduits have been developed to overcome the recognized limitations and disadvantages of the gold standard, the nerve autograft. These alternatives involve both synthetic and natural polymers, often incorporating cells or biological agents. Furthermore, in recent years, acellular nerve allografts (ANGs) have emerged as potential substitutes for traditional conduits [1]. This study aimed to compare the regeneration potential of different strategies in a long-gap defect in rats.
Methods: 24 adult Wistar rats underwent a 15 mm sciatic nerve grafting procedure using three different strategies: (i) the clinically used collagen conduit Neuragen® (NG); (ii) a decellularized nerve allograft (ANG) generated by a previously optimized protocol [2]; and (iii) the autograft approach (AUTO). Following 16 weeks, the animals were subjected to neurophysiology and then euthanized for histological (histochemistry & immunohistochemistry) and muscle morphometric analyses.
Results: Electrophysiological analyses showed more signs of reinnervation within AUTO and ANG groups as compared to NG. Histology (HE) revealed an active regeneration process, with axonal growth reaching the distal nerve stump in all treated animals. The MCOLL histochemical method demonstrated a higher level of remyelination in the AUTO and ANG groups compared to the NG group at both the medial and distal segments of the graft. Additionally, the NG group exhibited more collagen content instead nerve regeneration within the endoneurial compartment, particularly in the distal section. Nerve regeneration was confirmed by the high positive reaction for S-100 and NFL in both AUTO and ANG groups which were superior than NG group. Muscle morphometry was in line with electrophysiological and histological analyses confirming more muscle trophism when autografts or ANG were used.
Discussion & conclusions: In conclusion, these findings suggest that the decellularized allograft resulted in superior regeneration compared to the commercial collagen conduit in 15 mm gap defects, being comparable but not superior to the gold standard autograft group. Further studies using new functionalization strategies within acellular nerve grafts are needed to provide the necessary structural and molecular cues to overcome the efficacy of autograft technique.
Fundings: This research was financed by grant PI23/00337, funded by Instituto de Salud Carlos III (ISCIII), Ministry of Science, Innovation and Universities (Plan Estatal 2021-2023, and Plan de Recuperación, Transformación y Resiliencia). Co-funded by the European Union, Fondo Europeo de Desarrollo Regional ERDF-FEDER. Funded by grant CPP2021-009070 by “Proyectos de colaboración público-privada”, Plan de Investigación Científica, Técnica y de innovación 2021- 2023, Ministerio de Ciencia e Innovación, Unión Europea, Agencia Estatal de Investigación, España.

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C24 - Manufacturing of Hydrogel Microspheres for Three-Dimensional Culture of Mesenchymal Stem Cells and Obtainment of Their Secretome

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Introduction: Histological characteristics of hyaline cartilage, mainly its avascular nature, dictate its regenerative capacity. Currently, cell therapy is highly prevalent for treatments associated with this tissue. However, evidence has emerged suggesting that the sought-after effect is mediated by released molecules rather than the cells themselves [1]. For this reason, we aim to generate a secretome to enhance cartilage regeneration while circumventing the introduction of stem cells into affected individuals.
Methods: We developed a three-dimensional (3D) culture system using mesenchymal stem cells (MSCs), specifically human dental pulp stem cells (hDPSC). These cells are encapsulated in microspheres of a hydrogel composed of two polymers, alginate, and agarose, generated using a peristaltic pump-based system. These microspheres are maintained in a bioreactor for extended periods, allowing for the addition and withdrawal of culture medium and maintaining microspheres in suspension through agitation. The bioreactor also controls parameters such as temperature, humidity, and carbon dioxide (CO_2) percentage. During the culture period, hDPSC undergo chondrogenesis using a fetal bovine serum (FBS)-free differentiation medium [2], making it suitable for future clinical applications. We evaluated the elastic modulus of the hydrogel microspheres using a Zwick Roell elastomer. The conditioned medium collected from microsphere culture is utilized for various experiments to identify factors released by the cells in culture and assess their effects on tissue regeneration.
Results: The mentioned system allowed us to obtain fairly homogeneous microspheres in terms of size (diameter of 2.4 ± 0.5 mm) and cell density. So far, we achieved cell viability for up to 6 weeks in the differentiation medium. The elastic modulus increased in the presence of cells, with a 2.03- fold increase during differentiation and a 1.5-fold increase during proliferation.
Conclusions: The presented results suggest that the hydrogel does not hinder nutrient entry or gas exchange. Additionally, the cells are secreting some component that enhances hydrogel stiffness.

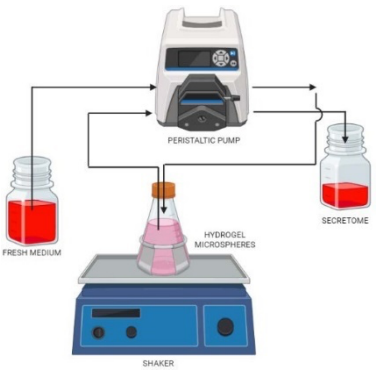

Figure 1. Schematic representation of the bioreactor.

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C25 - Fish scales as a sustainable source of biomaterials for artificial cornea development

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Introduction: The shortage of organ donors, such as the human cornea, results in long waiting lists and patients morbidity. Tissue Engineering emerges as an alternative capable of addressing this situation by developing artificial corneas able to replace allogeneic transplantation [1]. In this context, blue biomaterials, natural biocompatible products derived from waste in the fishing and fish farming industries, represent a promising resource for artificial cornea development [2]. Additionally, the use of these by-products as biomaterials could have positive socio-environmental impact. The objective of this study is to characterize a new sustainable model of human cornea developed using blue biomaterials (fish scales) as an effective and safe therapy, while also being environmentally friendly.
Methods: First, blue biomaterials were obtained from industrial by-products, which were then treated with a dual decalcification-decellularization process using Ana Morse methods. Subsequently, corneal epithelial cells were cultured, and the biomaterials were recellularized to generate artificial corneas. Finally, a histological characterization of the native biomaterials, decalcified-decellularized biomaterials, and the new sustainable artificial cornea model made with blue biomaterials were conducted.
Results: Our results demonstrated that the processing method described in this study was effective for the decalcification and decellularization of blue biomaterials, and properties of the scales were preserved. The studied biomaterials were partially biomimetic to the human cornea, showing great similarity in composition and structure. However, differences were found among the different types of scales. Recellularizing the biomaterial with corneal epithelial cells revealed proper adherence to the biomaterials, allowing for the development of functional artificial corneal models capable of partially reproduce the characteristics of the native cornea, while also being environmentally friendly.
Discussion & conclusions: Based on the aforementioned findings, fish scales blue biomaterials may represent an optimal resource for the generation of artificial corneas with potential therapeutic applications. Decalcified-decellularized scales, as well as the recellularized scales, shared important similarities with the native cornea and offered several advantages over other types of scaffolds. Blue biomaterials represent a promising sustainable source for constructing new artificial cornea models. Future studies should determine the therapeutic potential of artificial cornea models for treating severe pathologies affecting the corneal surface.

Acknowledgements: This work was supported by grants FIS PI23/00335 and ICI21/00010 (NANOULCOR), funded by Instituto de Salud Carlos III (ISCIII), Ministry of Science, Innovation and Universities (Plan Estatal 2021-2023, and Plan de Recuperación, Transformación y Resiliencia). Co-funded by the European Union, Fondo Europeo de Desarrollo Regional ERDF-FEDER, Programa Operativo FEDER Andalucía 2021-2027.

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C26 - Effect of functionalised gelatine-based hydrogels in corneal wounds reproduced in *in vivo* rabbit corneas

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Introduction: Persistent epithelial defects (PED) and chronic corneal ulcers are common ocular lesions that do not heal even after two weeks of treatment. The corneal surface becomes less sensitive and heals inadequately over time, which can result in corneal lysis and perforation. Although not challenging to diagnose, PEDs are challenging to treat. Non-invasive eye drops that aim to restore the normal structure and function of the epithelium are usually preferred, but severe cases may require surgical interventions such as tissue grafts or stem cell transplants. The present study focuses on the *in vivo* evaluation of a gelatine-based hydrogel functionalised with different compounds that could temporarily fill corneal defects and progressively release molecules that enhance healing without requiring surgical intervention.

Methods: 30 Female New Zealand white rabbits were randomly divided in 5 groups, 1 control group and 4 treatment groups. The treatment groups corresponded to the corneas treated with different photocrosslinkable gelatine-riboflavin phosphate (RFP) based hydrogels. The first treatment group corresponded to the non-functionalised gelatine-RFP hydrogel (H). The remaining three hydrogel- based treatments were functionalised with, Infliximab (H-Ab), an anti-TNFα antibody, autologous serum (H-AS) and human amniotic membrane extracts (H-HAMe). Corneal defects were reproduced *in vivo* by anterior superficial stromal keratectomies in rabbit models, the clinical aspects of wound closure were evaluated during the study period and, finally, processed samples were evaluated using immunohistochemistry and gene expression analysis. The extracted corneas were divided in 7-day processed corneas and 21-day processed corneas for the gene expression and immunohistochemistry analysis.

Results: The average healed epithelial area was greater in the control group than in the hydrogel- treated groups during the initial days (day 3, 4 or 5) of the experiment. Nevertheless, a few eyes in the control group failed to close, or even reopened by the end of the study, while all hydrogel-based treatments improved the wound healing response. A higher expression for the proliferation marker Ki67 was observed in corneas processed 7 days after surgery than in those processed 21 days after surgery, both at the mRNA and protein levels, regardless of the treatment received. Furthermore, Ki67 expression was higher in the control group at both times. All included treatments induced minimal signs of irritation on day 3 after surgery. The score was primarily due to redness of the palpebral conjunctivae, chemosis or, in some cases, discharge, most likely as a result of surgery-related inflammation and irritation. By day 4 and 5, all cases exhibited symptom improvement and the mean scores of the treatments were practically non-irritative. Gene expression results demonstrated for all hydrogel-based treatments a reduction in inflammation in 21-day extracted corneas in comparison to the control. The H-Ab treated group exhibited the most pronounced reduction in IL-1β expression at both the 7-day and 21-day extracted corneas. A significant decrease in α-SMA levels of the hydrogel treatments in comparison to the control treatment at both 7 and 21 days after the surgery was also registered. Concerning immunofluorescence, a disordered extracellular matrix and myofibroblasts were observed through α-SMA staining in all treatments to varying degrees. Although CK15 positivity, a putative marker of limbal epithelial stem cells, was present in all samples, it was noteworthy that eyes receiving H-Ab, H-AS and H-HAMe treatment 21 days post-surgery demonstrated significant clusters of undifferentiated CK15-positive cells in the basal limbal epithelium.

Discussion & conclusions: In response to the need for treatments for PEDs and corneal ulcers, the hydrogel developed from gelatine and RFP, functionalised with either blood-derived products, HAMe or an anti-inflammatory antibody, has proven to be a treatment that promotes re- epithelialisation, enhances tissue repair mechanisms by reducing fibrosis and controls the degree of inflammation to restore the overall ocular integrity.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

C27 - Novel monoclonal antibodies can distinguish Cripto-1 from Cripto-3 proteins. Clinical implications and potential new biomarkers

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Introduction: Cripto-1 (CR1) is an oncofetal protein required for implantation and expressed in the adult during wound repair, inflammation, and tumorigenesis. A second gene codes for a very homologous protein, Cripto-3 (CR3). Both proteins contain 188 amino acids and differ only in 6 residues. Heretofore, all available antibodies cannot discriminate between these two proteins, preventing any investigation on their differential contributions to cancer biology. Here, we present the generation, characterization, and clinical implications of highly specific antibodies (MoAbs) for these two proteins.
Methods: Different peptide fragments of either protein were used as antigens to generate mouse monoclonal antibodies (233 clones for CR1, 319 clones for CR3) that were selected by their targetprotein specificity through SPR and ELISA assays. Selected antibodies were used for immunohistochemistry on tissue arrays including normal tissues as well as cancers of the lung, breast, colon, ovary, and prostate. Antibodies were also used for Western blotting and ELISA characterization of human serum (breast cancer vs healthy controls).
Results: Monoclonal antibodies NCI 5G1-1 and NCI 5G11-2 were highly specific for CR1 and CR3,respectively. No crossreactivity was observed with the other protein. Immunohistochemical analysis of cancer specimens showed differential staining patterns, where some tumor cells expressed both proteins, others expressed only CR3, and still others show vascular endothelial cells stained for CR1 while tumor cells express CR3 (Fig. 1). In prostate, colon, and breast cancer, CR1 and/or CR3 protein expression correlates with clinical parameters, such as TMN_N, TMN_M, tumor stage, tumor grade, or PR expression. Lastly, both CR1 and CR3 interact with established binding proteins Nodal, GRP78 and Alk4 and competitively interfere with one another for targeted binding.
Discussion & conclusions: We describe for the first time the development of MoAbs that discriminate human CR1 from CR3, confirming that CR3 is translated into a protein in human cells,thus removing its pseudogene status. We also confirm CR1, and propose CR3, as cancer prognosis and severity markers.

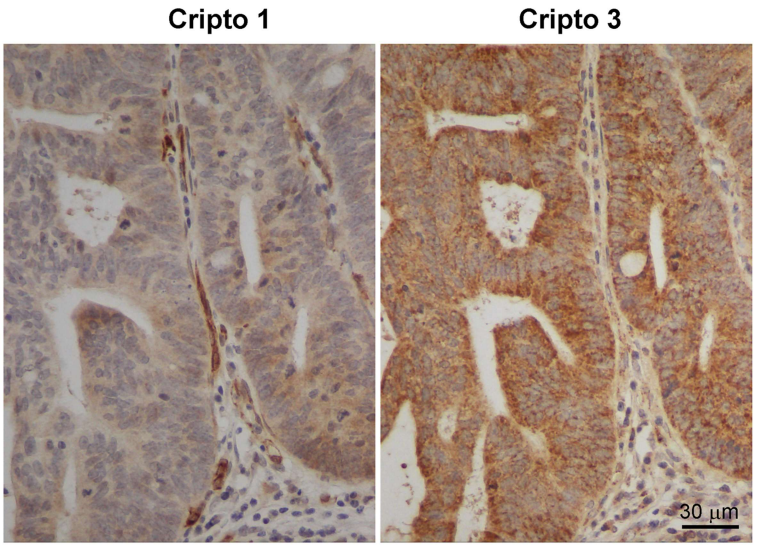

Figure 1. Serial sections of a colon adenocarcinoma specimen stained with antibodies against CR1 and CR3. Example of expression of CR1 invascular endothelial cells while tumor cells express CR3.

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C29 - Identification of Novel Prognostic Biomarkers in Pancreatic Adenocarcinoma: Integration of Histopathology in Clinical Practice

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Pancreatic cancer currently ranks fourth as a cause of cancer death in Europe and the United States, but it is anticipated to become the second leading cause of cancer-related mortality in the coming years. Pancreatic ductal adenocarcinoma (PDAC) accounts for up to 90% of all pancreatic tumor cases. Most cases are diagnosed at late stages due to challenges in early detection, resultingin a five-year survival rate of less than 10%. Research into serological and tissue biomarkers is integral to pancreatic cancer investigation, offering translational applications such as early diagnosis, precise prognosis, or potential therapeutic targets. Several studies have demonstrated a correlation between tissue expression of various molecules measured by immunohistochemistryand survival rates in pancreatic ductal adenocarcinoma patients, aiding in disease progression prediction and therapeutic alternative exploration. This communication aims to delve into this significant research line to underscore the relevance and necessity of integrating histopathology into clinical practice. We will focus on studying a wide array of molecules involved in cancer initiationand development, regulating key processes such as oxidative stress, inflammation, proliferation, cell cycle, and epigenetics. To deepen their prognostic value, Kaplan-Meier curves will be generated in a retrospective, observational analytical cohort of up to 41 pancreatic adenocarcinoma patients followed over 60 months. In parallel, correlation analysis of some of these molecules will be performed to explore possible interconnections in the expression levels of them. The results suggest the prognostic value and molecular interconnections of a diverse array of biomarkers detected by immunohistochemistry techniques, offering the potential for future studies to delve intotheir role in these tumors and evaluate their validity as therapeutic targets.

Keywords: Pancreatic ductal adenocarcinoma (PDAC); Prognostic biomarkers; Immunohistochemistry;Oxidative stress; Inflammation; Epigenetics.

C30 - Placenta Alteration in Women with a First-Episode Psychosis during Pregnancy

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New-onset psychosis in pregnancy poses risks to maternal and fetal health, characterized by a lossof reality contact. Psychotic disorders lack a formal definition but are identified through symptom domains (delusions; hallucinations; disorganized thought; among others). Prevalence estimates indicate 4.6 cases per 1000 persons for psychotic disorders and a yearly risk of 7.1 in 10,000 women for severe mental illness during pregnancy. Pregnant women with psychosis face elevatedrisks of adverse obstetric and neonatal outcomes, highlighting the need for a deeper understandingof the pathophysiological changes involved. The challenge for clinicians lies in balancing medication effects with maternal illness risks. Placental alterations due to psychiatric conditions have been documented, nevertheless limited studies explore placental changes in this vulnerable group, despite its crucial role in pregnancy physiology and maternofetal programming. Analyzing placental histopathological lesions in women with new-onset psychosis during pregnancy may provide insights into the implications of psychiatric disorders on maternofetal well-being. We haveaccomplished some histopathological evaluations addressing different pathways of expression. Among these, we highlight the importance of iron metabolism for maternal-fetal well-being. We notice iron overload, leading to oxidative stress and lipid peroxidation, altering cytoarchitecture of placenta and posing risks during pregnancy. Furthermore, we studied some alterations in placentalmorphology (Tenney-Parrker changes) and expression of hypoxic and apoptotic markers. Additionally, examination of oxidative stress markers in placental tissue reveals elevated gene andprotein expression, suggesting a potential role for oxidative stress in placental dysfunction associated with psychosis during pregnancy. This underscores the need for deeper understanding and clinical management strategies tailored to address these complexities.

Keywords: Placenta, Psychotic disorders, Tenney-Parrker changes and hypoxia.

Histology and Histopathology
From Cell Biology to Tissue Engineering

C31 - Protocol for evaluating histopathological responses in fish liver in aquatic systems

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Introduction: Coastal ecosystem are in environmental stress due to the impact of critical pollutants. To evaluate the effect of stressors agents, the use of bioindicator organisms and biomarkers has been implemented to establish a degree of environmental quality. The analysis of histopathological responses in the liver of fish has been used to evaluate the effect of environmental stress. The histopathological biomarker provides evidence of the impact of exposure to toxic compounds, allowing inferences about the health status of organisms and indirectly of aquatic systems. There are few standardized protocols for the semi-quantitative evaluation of the histopathological responses. Thus, it is important of the develop criteria for the quantification of the alterations, whichwill contribute to the management and monitoring of natural resources. Therefore, the objective wasto establish a protocol for the evaluation of the histopathological responses in the liver of fish. Methods: A total of 54 tissue sections were obtained from liver samples corresponding to six speciesof fish (sole, snapper, catfish, mojarra, anchovy and sardine), which were stained with hematoxylinand eosin. The functional unit of the liver was described and alterations in the tissue were recognized,which were classified into five categories of lesions. Photomicrographs of six optical fields, at 40x, were taken, for each tissue section. The photomicrographs were analyzed to quantify the area (μm^2)of the lesions using a specialized program (AxionVision, Rel. 4.8.2. SP2). Subsequently, apermutational analysis of variance (PERMANOVA) was made to evaluate differences in total areawithin and between the categories, and between the species, to enable a diagnosis of fish health. Results: The hepatic tissue structure of the species is constituted by parenchyma, blood and bileducts, hepatocytes, epithelial and endothelial cells. The lesions were recognized in 5 categories:circulatory alterations (I), inflammation (II), regressive changes (III), progressive changes (IV) and tumors (V). The evaluation of the area of the lesions within each category showed significantdifferences in the categories of circulatory alterations ($F=152$; $p=0.001$); inflammation ($F=161$;$p=0.001$); regressive changes ($F=377$; $p=0.001$); and progressive changes ($F=107$; $p=0.001$). Thisindicates that the lesions have different dissemination. The total area affected by category wascompared. The data showed significant differences between categories I and II, and categories III,IV and V ($F=145$; $p=0.001$). In the category III (regressive changes), a highest damaged area wasregistered in comparison with the circulatory alterations and inflammation, categories I and II,respectively, while progressive changes and tumors, categories IV and V, reported a smallerdamaged area. The affected area and dissemination can be associated with the importance factorproposed in standardized protocols, which assign lesions in the categories II and III, with importancefactors 2 and 3, implying a compromise in the condition of the organisms. Analyzing the lesioncategories between species, it was observed that sole, catfish, mojarra and snapper registered thegreatest area of damaged tissue compared to anchovy and sardine ($F= 22$; $p=0.001$). Discussion & conclusions: The identification of the liver tissue structure allowed the recognition andclassification of histopathological responses into lesion categories. Various studies have indicated that tissue alterations are associated with morphological, structural and metabolic changes in the organ, due to processes to isolate and eliminate stressors. The lesion categories with the bigger degree of damage were I, II and III, which coincides with the prevalence in research a global level. This suggests that, if the effect of environmental stress continues, histopathological responses mayreach an irreversible state. The 5 lesion categories were observed in the species, but the degree ofdissemination varied between them. This is associated with the environmental stress to which they are exposed and endogenous factors specific to each species. The biomarker provides evidence on the effect of environmental stress on the liver of fish in the system studied. It is an advantage to havestandardized protocols for the quantification of lesions, which will allow the development of useful algorithms in environmental biomonitoring.

C32 - Activation of intercostal muscle biomarkers in patients with chronic COVID-19 after pulmonary rehabilitation. A pilot study.

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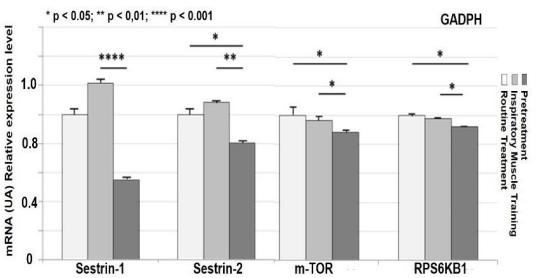
Introduction: Many people recovering from COVID-19 experience prolonged symptoms such as dyspnea, fatigue, exercise intolerance, and breathlessness [1]. We urgently need to identify safe and effective post-COVID-19 rehabilitative strategies and supervise exercise programs, including pulmonary rehabilitation (PR), to improve patients' health and quality of life [2]. The effects of inspiratory muscle training (IMT) in PR programs have proven effective in other diseases [3] but inpost-COVID-19 remains unclear, specifically due to persistent symptoms and post-acute consequences of SARS-CoV-2 infection. The characteristics and accessibility of the external intercostal (EI) muscle of patients with chronic respiratory pathology make it appropriate to obtain biopsies. The current study aimed to investigate the potential rehabilitative role of IMT on respiratory muscles. Methods: Six patients with previous SARS-Cov-2 infection (SpO2=93.6±1.9; FVC%=75.8±12.6; MIP%=78.7±31.4; ≥ 6 months after hospital discharge) were recruited at University Hospital of Soria (Spain) to cross-over study. Following baseline testing called pretreatment (PT), participants with chronic COVID-19 performed routine treatment (RoT) according to hospital clinical protocols (diaphragmatic breathing exercises, pursed lip breathing, chest expansion, and simple stretching exercises) for 6 weeks 2*day and later 12 weeks to the RoTpatients added the POWERbreathe® (PwB) KH1 (Biolaster, Spain) inspiratory muscle training (IMT) device at a workload of 60% MIP executing 2*30 breaths 2*day. After biopsies were performed of the fifth EI muscle (dominant side), the tissue samples were analyzed by light microscopy to determine conventional morphometry (hematoxylin-eosin staining), including minimum diameter (Dm) and fiber area (Ar) in cross-sections, and real-time polymerase chain reaction (RT-PCR; normalizing with house-keeping genes GAPDH) plus Western blot (Wb) to analyze the expression of the following muscle biomarkers: Sestrin (1 and 2), mammalian target ofrapamycin (mTOR) and Ribosomal Protein S6 Kinase B1 (RPS6KB1) following the manufacturer'sinstructions. Results: Morphometric study of muscle slice showed no significant differences between Dm or Ar on PT (Dm= 46±9 μm; Ar=2.4±1.1 μm^2), RoT (Dm= 48±8 μm; Ar=2.6±1.3 μm^2)and IMT (Dm= 51±10μm; Ar=2.8±0.9 μm^2; $p > 0.05$) sides. A significant decrease ($p < 0.05$) is shown between PT and RoT or IMT for mTOR, RPS6KB1, and Sestrin 2. Also, Sestrin 1 showeda significant decrease ($p < 0.05$) PT vs. IMT and a substantial trend ($p > 0.05$) to decrease in PT compared to RoT. Western blot analyzes of subcellular fractions showed a moderate increase in Sestrin 1, Sestrin 2 and mTor in the two respiratory interventions (RoT and IMT) compared to baseline. Discussion & Conclusion: Sestrin (1 and 2), mTOR and RPS6KB1 muscle biomarkers are regulators linked to energy metabolism, antioxidant power, and mass that are altered in states of muscle damage such as chronic COVID-19. In IE muscles after RP (RoT or IMT), it it is suggested that muscle damage could be neutralized due to the expression changes in the biomarkers analyzed. Our results would indicate the presenceof repair processes that would explain the possible improvement in their functional properties.



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C33 - Description of the Presence and Role in the Local Immunopathogenesis of Tertiary Lymphoid Structures in Non-ObeseDiabetic (NOD) Mice at Different Stages of the Disease.

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Introduction: Tertiary lymphoid structures (TLS) comprise aggregates of immune cells,notably B lymphocytes, within a network of fibroblasts interacting with T lymphocyte areas. These structures facilitate direct communication with neoantigens, enabling dendritic cells to interact with activate T lymphocytes. Positioned precisely where pathology takes development,TLS hold significant importance in disease pathogenesis. In neoplastic diseases, a positive correlation with subsequent immunotherapy response has been observed. Conversely, in autoimmune diseases such as Type I diabetes mellitus, the stimulation and formation of these structures lead to increased production of auto-antibodies and subsequent tissue damage. Despite the fact that there are autoimmune pathologies in which these structures have been extensively described, in the case of Type I diabetes mellitus, there are still many unansweredquestions. The non-obese diabetic (NOD) mouse model is extensively used to study Type I diabetes mellitus. In these mice, the disease develops due to leukocyte infiltration into pancreatic islets, resulting in insulinitis, thus allowing the study of pathogenesis and its extrapolation to human conditions.
Materials and Methods: Thirty female NOD/ShiLtJ mice aged 4-5 weeks were housed in groups of four, fed standard chow, and provided water ad libitum.Three groups (G) of 10 mice each were formed: G1euthanized at 5 weeks, G2 at 11 weeks, and G3 at 20 weeks. Pancreatic samples were collected during necropsies and fixed in 4% paraformaldehyde for histochemical tests such as Masson's trichrome,immunohistochemistry (including TUNEL), and multispectral immunofluorescence to establish a panel for characterizing and describing the development and presence of TLS. Different cellular localization markers have been employed, such as anti CD20, anti-CD19, anti-CD138, to phenotype B lymphocytes, along with a panel of biomarkers for tertiary lymphoid follicles consisting of anti-CXCR5, anti-CD21, and anti-PNAd. Therefore, a histopathological study was conducted using H&E staining to determine, through a semi- quantitative analysis, the severity of inflammatory infiltration at different stages.
Results: Preliminary analyses indicate that the presence and degree of TLS maturation exhibited a varying trend throughout pathogenesis. Differences were also observed in fibrosisdeposits within Langerhans islets, as well as TUNEL technique positivity.
Conclusions: Understanding the presence and formation of tertiary lymphoid structures in thepancreas of NOD mice and relating it to local immunopathogenesis at different stages of the disease could be one of the exacerbating factors of the pathology, as well as disease maintainers.

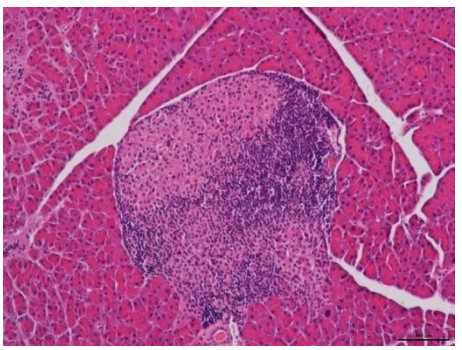

Figure 1. Moderate to severe inflammatory infiltration in the islet of Langerhans of NOD mouse at 11 weeks of age.

C34 - Effect of ischemia-reperfusion of the gastric mucosa on lung, a remote organ

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Introduction: Ischemia-reperfusion (I/R) injury in the gastrointestinal is common in critically ill patients and can cause the translocation of bacteria and inflammatory molecules (cytokines) to thesystemic circulation. This can result in septic shock and/ or systemic inflammatory response syndrome (SIRS), remote organ damage and death [1]. The study of remote organ damage has been focused on intestinal I/R models, and its effect in kidney, brain, liver and testis. However, recent studies from our I/R model of the gastric mucosa show high levels of oxidative stress and increase in apoptosis [2]. These results suggest that remote organ damage may can occur in our gastric mucosa I/R model.
Methods: Male Wistar rats (250-350 g), divided into 4 groups (n=5): rats with I/R subjected to gastricmucosa ischemia (by occlusion of the gastric arteries) for 60 or 120 min, then occlusion was removed to allow reperfusion for 24 h; sham operated rats underwent surgery without occlusion ofthe gastric arteries; and finally control rats, which did not undergo surgery. Biopsies of the stomach body and left lung were removed, processed by histological technique, and stained with hematoxylin and eosin, to perform a preliminary histopathological analysis.
Results: In gastric mucosa, under I/R conditions of 60 min/24h epithelial cells with pyknotic nuclei,apparent scarring areas, loss of glandular structure and presence of cellular debris were observed.Finally, under I/R conditions of 120 min/24h apparent areas of scarring, strong eosin staining of mucinogen and recovery of the glandular structure were observed.
In the lung, under I/R conditions of 120min/24h apparent loss of surfactant was observed in the terminal bronchioles, strong eosin staining in the bronchiolar epithelium, and infiltration of polymorphonuclear cells in alveoli and interstitial space between alveoli.
Discussion & conclusions: Damage to the gastric mucosa under I/R conditions for 60min/24h is similar to the damage observed in our previous studies [2]. However, under I/R conditions of 120min/24h, apparent signs of recovery are observed.
Changes in lung are similar those reported in intestinal I/R models [3]. Our results suggest that longer ischemia time can lead to a local (or systemic) tissue adaptation to damage. Finally, the present study suggests that I/R of the gastric mucosa induce changes in remote organs such as the lung.

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Oral Presentations

Sessions of Thursday 12th (16:30h -18:00h)

Oral 7 – Histology and Reproduction

Moderators:
Ester Beltrán Frutos
María de los Llanos Medrano López-Tello

Oral 8 – General Histology II

Moderators:
Jesús Chato Astrain
Juan Antonio López Villodres

Histology and Histopathology
From Cell Biology to Tissue Engineering

C35 - Histological determination of a new zone in the intratesticular pathways of the seminiferous tubules of the adult Syrian hamster.

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Introduction: The intratesticular pathways are defined as the region from the final part of the seminiferous tubules to the rete testis and commonly divided into the terminal segment (valve zone), the straight tubule, and the rete testis. This region has recently been characterized by having cells with a high differentiation potential. Although this region has been the subject of past investigations, a clear understanding of its components is lacking. Existing studies have not reached a consensus on the cell populations present or the phenotypic variations among them. Aim: To perform a tissue evaluation of the zones terminal segment and straight tubule of the Syrian hamster testis, and phenotypically characterize the Sertoli-like cells within these regions to localize the cells with the greatest differentiation potential.

Methodology: Testes from four young, fertile Syrian hamsters were used. The samples were processed for light and transmission electron microscopy (TEM). Routine hematoxylin-eosin (H-E) staining, lectin histochemistry, and examination of semi-thin sections were employed for the histological description of these zones. In addition, an immunohistochemical study was conducted on Sertoli-like cells using vimentin marker to identify every Sertoli cells, cyclin D1 as a marker of undifferentiated cell proliferation, and desmin and cytokeratin 8/18 (CK 8/18) as markers of immature Sertoli cells. A qualitative study was performed to determine the expression of desmin, vimentin and CK8/18, while cyclin D1-positive cells were semi-quantitatively assessed as a proportion of the total cells observed in these regions.

Results: Under light microscopy, 4 zones were distinguishable: the final part of the seminiferous tubule, formed by an epithelium of Sertoli cells, spermatogonia, and some spermatocytes; the valve, consisting only of Sertoli-like cells with their processes directed towards the straight tubules; the transitional zone, made up of cells with a flattened morphology; the straight tubule, composed of cells with a cubic morphology, and which tubular diameter is smaller than the previous portion. According to the immunohistochemical study, from a qualitative point of view, Sertoli cells in the final portion were strongly positive for desmin but negative for CK 8/18. In the valve, Sertoli-like cells were very slightly positive for desmin and negative for CK 8/18. In the transition zone, as well as in the straight tubules, flattened cells were found to be positive for CK 8/18 but negative for desmin and sometimes for vimentin. The semi-quantitative study of cyclin D1 protein revealed a significant increase ($p < 0.05$) in the percentage of positive Sertoli-like cells from the final portion (56.8%) to the valve (73.8%). This expression significantly decreased in the transition zone (31.9%), with no positive cells detected in the straight tubules. Lectin affinity showed a different pattern between the valve and the transition zone. TEM confirmed the zones and showed ultrastructural differences between Sertoli-like cells in the final portion and the valve, as well as the presence of germ cells undergoing apoptosis. Cells in the transition zone showed few cell organelles.

Conclusions: The terminal segment of the Syrian hamster seminiferous tubules harbors a newly described zone, the transition zone. This area begins to present cells an epithelial character similars like to straight tubules, although retaining proliferative capacity. The valve, on the other hand, shows vimentin-positive Sertoli cells with a high proliferation rate, which is lower in the final portion of the seminiferous tubule. This indicates that there is continuous dedifferentiation of Sertoli cells from the seminiferous tubule to the transition zone, followed by epithelial differentiation towards the rete testis. The somatic epithelial cells of the terminal segment, and especially of this new zone, suggest a potential reservoir of undifferentiated somatic cells and raise the possibility of their application in regenerative medicine and testicular cell therapy.

C36 - The crucial role of ZP1 during folliculogenesis: impact on zona pellucida formation and fertility in rabbits (*Oryctolagus cuniculus*)

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Introduction: During ovarian folliculogenesis, an extracellular glycoprotein matrix known as the zona pellucida (ZP) is synthesized surrounding the oocyte. The ZP is involved in assorted physiological processes, including the protection of oocytes and embryos, fertilization, and early embryo development. Across different eutherian mammalian species, the composition of the ZP is species- specific, comprising three or four glycoproteins. In humans and rabbits, ZP consists of four distinct glycoproteins (ZP1, ZP2, ZP3, and ZP4), whereas in mice, it comprises only three (ZP1, ZP2, and ZP3). Prior gene ablation studies have highlighted the structural significance of ZP4 in rabbits, where its ablation results in subfertility [1]. In contrast, *ZP2* ablation in rabbits does not affect ZP formation or fertility [2]. *Zp1* ablation in mice impairs the formation of the ZP, which is unable to protect the developing embryo, resulting in reduced litter size [3]. This study delves into revealing the functional significance of ZP1 in rabbits, in which all four ZP glycoproteins are present. Methods: *ZP1*-null rabbits were generated using CRISPR-Cas9 technology following previously described methods [1]. A 26-nucleotide deletion within the first exon of *ZP1* gene disrupted the open reading frame at the 16th amino acid residue, generating a knockout (KO) allele. Two KO female rabbits were used in the present study. Ovaries were fixed in 4% formaldehyde for 24 h and embedded in paraffin for histological analysis. Sections of 5 µm thickness were prepared and stained with Hematoxylin and Eosin following standard procedures. Results: Following several crossbreeding attempts, ZP1-heterozygous ($ZP1^{+/-}$) females resulted fertile, having litter sizes comparable to those of wild-type (WT) animals (Table 1). In contrast, ZP1- KO ($ZP1^{-/-}$) females demonstrated complete infertility, failing to produce litter after four breeding crosses (Table 1). Histological analysis revealed that KO ovaries exhibited normal morphology, although both preantral and antral follicles contained oocytes lacking the ZP coat (Figure 1).

Table 1. Litter size between different female genotypes and the number of crosses and females.

Female genotype	Mean ± SE	n crosses (n females)
WT	6.6 ± 0.9	5 (4)
$ZP1^{+/-}$	6.5 ± 0.8	10 (4)
$ZP1^{-/-}$	0.0 ± 0.0	4 (2)

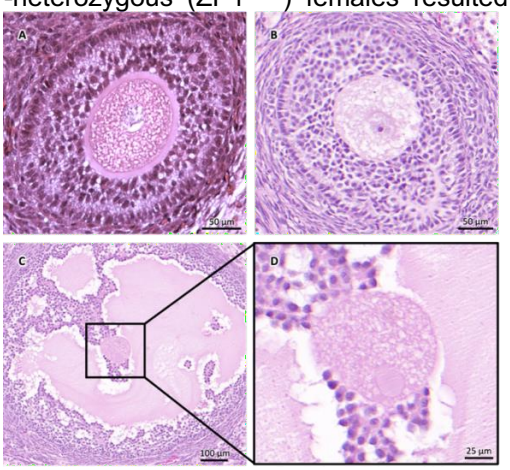

Figure 1. Histological sections of WT (A) and $ZP1^{-/-}$ (B- D) ovaries with preantral (A, B) and antral follicles (C). No ZP was observed in the KO oocytes. D is the magnified region framed in C

Discussion & conclusions: The novel findings of this research suggest a critical role for ZP1 in the formation of the zona pellucida during ovarian folliculogenesis. In *ZP2*- and *ZP4*-null rabbits, the ZP does form, although its thickness and *in vivo* functionality are compromised [1,2]. Hence, in the absence of a comprehensive understanding of the role of ZP3, we conclude that ZP1 is required for zona pellucida formation and rabbit fertility.

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C37 - Expression of the oxytocin receptor in sperm, testis and epididymis of horses

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Introduction: Leydig and Sertoli cells in the testes and the muscular layer of the epididymis are known to express the oxytocin receptor. Recently, the oxytocin receptor has also been identified in human sperm, suggesting that it could modulate their activity and be used as a marker of male fertility [1]. Despite this, whether this receptor is also present in horse sperm has not been interrogated, nor whether is also expressed in their testes and epididymis.
Objectives: For this purpose, the presence of the oxytocin receptor in horse sperm was determined by Western blot after protein extraction. On the other hand, testicular and epididymal tissues from castrated horses were fixed in Bouin solution, processed and subjected to immunohistochemistry for the oxytocin receptor. Localization of the oxytocin receptor in horse sperm was also determined on extensions with immunocytochemistry. The presence of the oxytocin receptor in horse sperm was confirmed with a 55-kDa band in blots. By confocal microscopy, this receptor was revealed to localize in the equatorial segment and in connecting of horse sperm. By brightfield microscopy, Sertoli and Leydig cells in testes were positive for the oxytocin receptor, as too were spermatocytes and spermatids. Regarding the epididymis, the muscular layer, and the perinuclear region of both principal and basal cells were also positive for the oxytocin receptor. In the epididymal lumen, apical blebs showed heterogeneous positivity.
In conclusion: a) horse sperm have the oxytocin receptor, which comes from spermatogenesis; and b) the presence of this receptor in apical blebs of epididymal lumen suggests another source of the oxytocin receptor during sperm maturation.

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C38 - Immunofluorescence assessment of Zonadhesin (ZAN) during mammalian spermatogenesis and in mature sperm

Hernández-Falcó M.[1], López-Botella A.[1], Ferrer-Cortés N.[1], Díaz-Fuster L. [1], Robles- Gómez L.[1], Sáez-Espinosa P.[1], and Gómez-Torres M.J.[1]

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Introduction: It is well known that acrosomal proteins are required to carry out the fertilization process. The synthesis of this acrosomal vesicle occurs during spermiogenesis, a phase in which spermatids undergo extensive cellular remodeling until they become spermatozoa. These spermatozoa are released into the epididymis where a maturation process is carried out. The protein zonadhesin (ZAN) belongs to the set of proteins contained in the acrosome. ZAN mediates the interaction of the spermatozoon with the zona pellucida, playing a key role in gametes recognition. The aim of this study was to determine the localization of ZAN during mammalian spermatogenesis and in mature sperm.
Methods: Testis samples and fresh ejaculate from boar and golden hamster were use. Paraffin- embedded tissue sections (4-7µm thickness) were obtained using a HM340 E microtome and placed on slides. Before staining process, tissue sections were deparaffinized with xylene and rehydrated with a decreasing series of ethanol to distilled water. Tissue sections and fresh ejaculates were washed and permeabilized with 0.2% (w/v) Triton X-100 during 10 min. The non- specific binding was blocked with 2% (w/v) bovine albumin serum during 1 hour for testis sections and 10 min for fresh ejaculate. Then, samples were incubated overnight at 4ºC with a direct ZAN antibody (1:100) conjugated with Cy^3. After washing, samples were mounted using Fluoroshield™ with 4′,6-diamidine-2′-phenylindole dihydrochloride. Finally, testis sections and fresh ejaculated were analyzed by Confocal Laser Scanning Zeiss LSM 800 Microscope.
Results & Discussion: ZAN was detected similarly in testis sections of both species. ZAN was first localized at the periphery of primary spermatocytes. Consequently, the protein was concentrated in a region of the secondary spermatocytes, which is consistent with the genesis of the acrosomal vesicle from the Golgi apparatus. A heterogeneous topographical distribution of ZAN was also observed in elongated spermatids. In contrast to the literature [1], we observed ZAN at earlier stages of spermatogenesis, perhaps owing to the target sequence of the antibody used. Even though ZAN has been classified as an acrosomal protein, it was found to be present in both pre- and post-equatorial regions of mature mammalian sperm cells. Additionally, it was observed in some spermatozoa exclusively in the post-equatorial region, which agree with a typical localization of reacted sperm.
Conclusions: For the first time, ZAN was detected at early stages of spermatogenesis.

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C39 - SPAM1 localization by fluorescence microscopy in fresh, capacitated and reacted porcine sperm cells

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Introduction: One of the molecules involved in the egg fertilization process is sperm adhesion molecule-1 (SPAM1). SPAM1 participates in different events, being vital its involvement in the cumulus oophorous dispersion due to its hyaluronidase activity at neutral pH. Nonetheless, hyaluronidases perform other functions rather than the catalysis of hyaluronic acid, being multifunctional proteins. Related with this, it has been suggested that SPAM1 performs a secondary ZP binding after the acrosome reaction. Due to its importance, the aim of this study was to characterize porcine SPAM1 protein in different sperm physiological conditions depending on their acrosomal status: acrosome intact non-capacitated spermatozoa, in capacitated sperm, and in acrosome reacted sperm cells after the induction of the acrosome reaction.
Methods: A total of 10 seminal samples were obtained from mature fertile tested Pietrain boars. The samples were divided to study different physiological conditions: non-capacitated sperm (NCS), selected after one-hour capacitation by *swim-up* technique (CS), and after the induction of the acrosome reaction next to capacitation (ARS). A co-staining was performed to assess SPAM1 localization in acrosome intact and acrosome reacted cells. Sperm cells were overnight incubated at 4 °C with an anti-SPAM1 antibody (1:100) produced in rabbit (Sigma-Aldrich, San Luis, USA). Then, smears were incubated in darkness for 1 hour with a secondary anti-rabbit antibody conjugated with Cy3 (1:100, Jackson ImmunoResearch, Ely, United Kingdom). Afterwards, the samples were incubated with the lectin PNA-FITC (Vector Laboratories, Burlingame, CA). Finally, the smears were assembled with Fluoroshield™ with DAPI (Sigma-Aldrich®, St. Louis, MO, USA). Appropriate negative control experiments were performed. For each condition, 200 cells were evaluated using a Zeiss LSM 800 Confocal Laser Scanning Microscope (Zeiss, Oberkochen, Germany). SPAM1 labeling was assessed in acrosome intact sperm cells from NCS and CS and in acrosome reacted cells from ARS. SPAM1 staining patterns were quantified as percentages (%). Results: The analysis of SPAM1 distribution in porcine sperm revealed three different staining patterns. Two staining patterns were found in acrosome intact spermatozoa belonging to NCS and CS conditions. In P1, we found SPAM1 labeling the pre-equatorial region, while an absence of labeling in the whole sperm head characterized P2. P1 was significantly higher in both physiological conditions (96.79 and 86.28 %, respectively; $p<0.01$). Regarding acrosome reacted cells from ARS condition, two staining patterns were found again (P2 and P3). P3 was characterized by SPAM1 labeling in the post-equatorial region. In this context, both patterns were present in the acrosome reacted sperm population, being none of them significantly higher (47.65 and 52.35 %, respectively; $p=0.88$).
Discussion & conclusions: SPAM1 has been designated as the main sperm protein with hyaluronidase activity, which is necessary to across the cumulus layer and reach the oocyte's zona pellucida. This could explain the staining localization of SPAM1 in the pre-equatorial region in acrosome intact cells in NCS and CS. Furthermore, some authors suggest an important role of SPAM1 in the secondary junction involved in sperm-zona pellucida binding after the acrosome reaction. Although we found SPAM1 relocalization to the post-equatorial region (P3), we also found an absence of SPAM1 labeling after the acrosome reaction (P2) in ARS. This could be due to its disappearance, or it may be that SPAM1 is masked, and the antibody cannot bind to it. As a result of losing SPAM1 from the post-equatorial region after the acrosome reaction, secondary binding to the ZP could be compromised, as cells that have failed to retain SPAM1 could not have the ability to bind to it. Thus, SPAM1's role after the acrosome reaction should be addressed in future research.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

C40 - Zonadhesin characterization in *Oryctolagus cuniculus* spermatozoa during capacitation and acrosome reaction

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Introduction: Fertilization is a complex and orchestrated process involving the adhesion, binding and fusion between male and female gametes. In this context, zonadhesin (ZAN) is implicated in the species-specific *zona pellucida* adhesion. This multiple-domain transmembrane protein has been consistently found in the sperm anterior head of a number of mammalians, including species of commercial interest or research animal models such as the rabbit (*Oryctolagus cuniculus*) [1]. However, the specific location of ZAN after key sperm physiological events such as capacitation or acrosome reaction is unknown. The aim of this study was to characterize ZAN protein in rabbit spermatozoa subjected to *in vitro* conditions of sperm capacitation and acrosome reaction.

Methods: Semen samples were obtained from rabbits housed in the Animal Facility of the Universidad de Murcia (Murcia, Spain). After evaluation of semen parameters, a total of seven samples were included in the study and divided into three physiological conditions: non-capacitated (NC), selected after capacitation (C) and after induction of acrosome reaction from capacitated spermatozoa (AR). Selection of capacitated spermatozoa was performed after incubation in TALP medium supplemented with 5mg/mL albumin using the swim-up technique. The acrosome reaction was induced by incubation with the ionophore A23187. After the achievement of each condition, NC, C and AR sperm samples were fixed in 2% paraformaldehyde diluted in PBS and the immunolabeling protocol was then performed. The spermatozoa were incubated overnight with a primary anti-ZAN antibody (1:100) produced in rabbit after a permeabilization with Triton X-100. Subsequently, the cells were incubated with a secondary anti-rabbit antibody conjugated to Cyanine ™3 (1:100). To assess the distribution of ZAN depending on the acrosomal status, samples were co-stained with the lectin Peanut agglutinin conjugated to fluorescein (PNA- FITC). Chromatin labeling was then performed using 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI). In this regard, ZAN distribution patterns were identified in acrosome intact NC and C populations and in acrosome reacted cells in AR sperm by Confocal Laser Scanning Zeiss LSM 800 Microscope. Appropriate negative control experiments were conducted to validate the specificity of primary antibody. Finally, statistical analysis was performed by IBM SPSS Statistics19.0. The aforementioned experimental design was developed at the Departamento de Biotecnología of the Universidad de Alicante (Alicante, Spain).

Results: The microscopical analysis showed five consistent ZAN distribution patterns: absent fluorescence (P1), fluorescence in the pre-equatorial region (P2), fluorescence in the pre-equatorial and post-equatorial regions (P3), fluorescence in the post-equatorial region (P4) and, fluorescence in the equatorial and post-equatorial regions (P5). In NC intact spermatozoa, the most-represented patterns were P3 (50,45%) and P2 (46,05%). However, after capacitation, intact spermatozoa showed mostly P2 (76,79%), followed by P3 (21,72%). The changes observed were statistically significant between NC and C for both patterns ($p<0,05$). In AR, acrosome-reacted cells showed specially P5 (39,57%) and P1 (38,37%). P1, characterised by the absence of fluorescence for ZAN, was recorded in less than 5% of NC and C spermatozoa, thus showing a significant increase after the acrosome reaction. Finally, P4 was observed only in AR condition, reaching 22.07% of the analyzed cells.

Discussion & conclusions: Our findings reported significant changes in ZAN distribution following essential events in sperm physiology such as capacitation and acrosome reaction. Moreover, this study reveals the presence of ZAN in membrane regions other than pre-eqautorial, such as the equatorial and post-equatorial regions. This would provide an opening for new functions for ZAN in sperm physiology, in addition to its role in the binding of the *zona pellucida*. Financial support: project PID2021-123091NB-C22 funded by MCIN/AEI/10.13039/501100011033 y por FEDER Una manera de hacer Europa.

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C41 - Inmmunolocation of oocyte receptor JUNO after vitrification and parthenogenetic activation

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Introduction: Fertilization is a complex process that involves multiple steps to ensure the successful union of sperm and oocyte, leading to the formation of a zygote. JUNO, a receptor protein on the oocyte membrane also known as folate receptor 4 (Folr4), specifically binds to IZUMO1, a protein on the surface of the sperm. This binding is crucial for the initial recognition and attachment between the sperm and the oocyte, facilitating membrane fusion, preventing polyspermy, and initiating oocyte activation. Although evidence exists for the localization of JUNO in human oocytes, no data are available on how vitrification and parthenogenetic oocyte activation could affect JUNO. Therefore, the objective of this study was to analyze the presence and localization of JUNO in human oocytes at various stages of maturation.
Methods: A total of 50 oocytes from patients attended in the Unidad de Reproducción Asistida Humana of the Hospital Universitario y Politécnico La Fe (Valencia, Spain) were decumulated and divided in four groups: 12 germinal vesicles (Group 1), 13 metaphase II matured in vitro (Group 2), 11 metaphase II matured in vitro and parthenogenetically activated (Group 3), and 14 vitrified germinal vesicles, matured to metaphase II and parthenogenetically activated (Group 4). All the oocytes from different groups were fixed in 2% paraformaldehyde. Then, oocytes were incubated with a primary Folr4 antibody (1:200) produced in rabbit overnight. After that, the cells were incubated with the secondary antibody anti-rabbit conjugated with Cyanine™3 (1:100) for 90min. Then, for chromatin staining, we used 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI). Negative controls were performed. This experimental part was performed at the Departmento de Biotecnología of the Universidad de Alicante (Alicante, Spain). Finally, the oocytes were analyzed by Confocal Laser Scanning Zeiss LSM 800 Microscope and the statistical analysis was performed by IBM SPSS Statistics19.0.
Results: The immunolocalization results showed that the JUNO receptor presented two localization patterns. The first pattern (P1) was associated with the presence of JUNO in the oolemma region while the second pattern (P2) was characterized by the presence of JUNO throughout the cytoplasm. In relation to the distribution of JUNO localization patterns in the different groups of oocytes analyzed, it should be noted that P1 was significantly predominant in groups 1, 2 and 3. While, P2 was significantly predominant in the group of oocytes that they had been vitrified prior to maturation and activation (Group 4). Furthermore, regardless of the oocyte group, Juno showed a polarized localization. Specifically, these proteins were expressed more intensely on the opposite side (vegetal pole) where the metaphase plate is located (animal pole).
Discussion and conclusions: Based on the results obtained, vitrification affects the distribution of the JUNO receptor and therefore this could negatively affect the fertilization process. This could be one of the reasons why after vitrification it is recommended to use the intracytoplasmic sperm injection (ICSI) technique. Furthermore, for the first time the presence of JUNO after parthenogenetic activation in human oocytes has been corroborated. These data coincide with studies in mouse oocytes where it was observed that after parthenogenetic activation JUNO remained in the oolemma, while if fertilization took place naturally JUNO was eliminated through the cortical granules [1]. Understanding JUNO's function not only provides insights into the fundamental aspects of fertilization but also has significant implications for addressing fertility issues and developing new contraceptives.

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C42 - Evaluation of the changes generated in the cardiac nodes after an acute spontaneous myocardial infarction in humans and pigs. A morphometric and histological study.

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Introduction: The sinoatrial node is responsible for the intrinsic electrical activation that in mammals leads to coordinated rhythmic contractions of the heart, where it is then distributed through the atrial tissue to the atrioventricular node [1, 2]. The purpose of this study was to describe histologically and morphometrically the components and cells in cardiac nodes altered by myocardial infarction and compare them with normal tissues in humans and pigs.

Methods: We analyzed ten human hearts, ten from pigs who died from myocardial infarction.Histological section thickness of 5 µm were obtained with a microtome and stained with hematoxylin-eosin and Masson's trichrome. A 4x magnification was used to measure the node parameters and a 10× or 20× magnification was used to measure the cell parameters.

Results: In infarcted humans the sinoatrial node showed a rounded shape and in infarcted pigs its shape was ovoid. The atrioventricular node presented a ovoid shape in both species.An increase in the percentage of collagen fibers was observed within the sinoatrial node of infarcted humans (58%) compared to normal nodes (52.6%); Likewise, the sinoatrial node ofinfarcted pigs showed a higher percentage of collagen fibers (63.4%) compared to normal animals (45%), which leads to a decrease in the percentage of cells. In the atrioventricular node, a decrease in the percentage of cells was observed in infarcted humans (35.1%) compared to normal humans (45.6%) and the same was found in infarcted pigs (19.9%) compared to the percentage of cells in the interior of the node in normal pigs (20%). The minimum diameter of P cells in sinoatrial node was greater in humans than in pigs ($p=0.007$).The area and diameters of surrounding cardiomyocytes were significantly larger in pigs thanin humans ($p<0.001$). The area, diameters and roundness of P cells, T cells and cardiomyocytes in the atrioventricular were significantly greater in pigs than in humans ($p<0.001$). In the sinoatrial node of infarcted humans and pigs, the P cells were hypertrophicand, in the T cells, there was a decrease in size, compared to control cases. A decrease in size was observed in the P cells of the atrioventricular node in infarcted humans and pigs. The T cells of the node in humans showed a decrease in size and in pigs hypertrophy whencompared with control cases. In humans, cardiomyocytes showed hypertrophy and in pigs aslight decrease in size when compared to normal hearts. When using the desmin stain to improve the identification of the nodal cells, we observed that there was difficulty in distinguishing nodal cells from cardiomyocytes in hearts that have suffered a myocardial infarction in the sinoatrial node of both species and in the atrioventricular node of humans, observing a greater difference in intensity between nodal cells and cardiomyocytes. in the atrioventricular node in pigs.

Discussion & conclusions: Myocardial infarction causes alterations in the tissue and cellularstructure of the cardiac nodes in humans and pigs that lead to the possible development of cardiac arrhythmias that lead to sudden death.

Keywords: sinoatrial node, atrioventricular node, myocardial infarction, humans, pigs

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C43 - NETs formation in response to the use of Mesoporous Silicon Microparticles as an adjuvant for a SARS-CoV-2 vaccine in mice

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Introduction: For a century, adjuvants have been necessary for efficient vaccines in humans. Aluminum hydroxide (alum) is the most widely used adjuvant, but new technologies are being investigated, which have shown to elicit a more targeted immune response. We are currently exploring the effect of Mesoporous Silicon Microparticles (MSMPs) when used as a potent adjuvant for a SARS-CoV-2 vaccine in mice. Neutrophils are the first immune cells recruited at the injectionsite. In response to several stimuli, they can release cellular DNA together with granular material, the so-called neutrophil extracellular traps (NETs). These web-like structures are decorated with histones, such as the citrullinated Histone 3, and neutrophil granule proteins such as myeloperoxidase (MPO), elastase, and cathepsin G [2]. In mice, NETs apparently play a role in alum-adjuvant immune responses to vaccines [3]. Our goal is to explore the response of neutrophilsto a MSMPs-adjuvant based vaccine and the formation of NETs both *in vitro* and *in vivo*.
Methods: For the in vitro experiments, human neutrophils were isolated from blood samples and plated on coverslips. They were subsequently treated with PBS, Alum or (4,9mg/mL) MSMPs for one and three hours. After that, cells were fixed in PBS-triton and immunostained with anti-MPO antibody (R&D) and a secondary antibody with Alexa Fluor 594 (Invitrogen). For chromatin staining, we used 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI). The sections were analyzed by Confocal Laser Scanning Zeiss LSM 800 Microscope. For the *in vivo* experiments, a total of 6 mice divided in three groups received intramuscular injections of PBS, alum + spike, and MSMPs + spike, respectively. 1 mouse from each group wassacrificed 4h later, and the other one after 24 h. Legs receiving the injections were fixed in formalinand embedded in paraffin. Serial 4 μm sections were obtained. In order to find the region of injection, 1 out of every 10 sections was stained with hematoxilin-eosin. Once located, serial sections were immunostained with the following primary antibodies: anti-neutrophil elastase (Abcam), and anti-MPO antibody (R&D).
Results: In the *in vitro* assays, a marked difference can be observed between the cells treated with MSMPs and Alum, both in the quantity of NETs formed, which are more abundant in the MSMPs treated samples, and in their dimensions. NETs are larger in the MSMPs treated cells and show amore scattered pattern. In most neutrophils where NETs are observed, the DAPI staining is eitherlost or faint.
In the case of muscle samples, a similar trend is observed. In the samples from mice immunized with MSMPs + spike, NETs are visible and abundant 4h after injection, and the quantity of neutrophils present in the infiltrate is high. 24h after injection, cell infiltrates are still visible but the number of neutrophils is reduced with respect to the 4h time point. The formation of NETs in the tissue is more evident 4h after injection. In the samples from mice immunized with alum + spike, we observe a large infiltrate in the site of injection which is larger at 4h than at 24h as well, and neutrophils are abundant but don´t seem to form as many NETs as with the previous treatment.
Our results could contribute to a better understanding of neutrophil responses and shed light on the use of MSMPs as new adyuvant in vaccine complex.

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C44 - Primary cilia function as a mechanosensor in the thyroid gland

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Introduction: The role of primary cilia (PC) in the thyroid gland remains unclear. Recently, some functions have been assigned to PC by several researchers, all of them associated with the biosynthesis of thyroid hormones either through an endocytic thyroglobulin (TG) pathway, basal autophagy alterations or a cathepsin mediated track of processing the TG [1]. Moreover, as our group have demonstrated, changes in the PC characteristics suggest a direct relationship betweenciliogenesis, follicular activity and follicular heterogeneity, and are related to pathological situations[2]. Considering the privileged localization of PC in the thyroid, projecting from the thyrocytes apicalsurface and immersed into the colloid, as well as the morphometrical variations that PC shows depending on colloid density, one of the functions of PC in the thyroid could be sensing the refillingof thyroid follicles. Therefore, the main objective of this project is finding out whether the sensing of mechanical stimuli plays a decisive role in the thyroid ciliogenesis, as happened in the kidney and the liver, where ciliogenesis defects induce the development of pathologies called ciliopathies. Methods: To achieve this objective, we analyzed the expression of different proteins related to thisfunction, such as polycystin-1 (PC1) and polycystin-2 (PC2) by immunofluorescence (IF), in both normal paraffin embedded thyroid sections and normal human thyroid follicular epithelial cells (Nthy-ori 3-1). Furthermore, we performed a double IF to test if these proteins are co-localized in the ciliary membrane. Then, we evaluated the expression of these proteins, in thyrocytes through molecular biology techniques, like *RT-PCR*. As positive control we used kidney, where the localization of these proteins in PC has already been proved. Results: In thyroid tissue sections, we have demonstrated by IF that PC1 and PC2 are expressed in human thyrocytes. The immunostaining pattern was similar for both proteins and, through double IF it was proved their colocalization with PC. These proteins, implicated in mechanotransduction processes, have also been identified in cultures of normal human thyrocytes, in which, we have confirmed by double IF their localization in PC. Moreover, we have evidenced the expression of PC1 and PC2 in cultures of Nthy-ori 3-1 cells at molecular level. Discussion & conclusions: In summary, these results support the hypothesis of the mechanosensory function of PC in the thyroid gland and raise the need to assess their relationship to ciliogenesis in order to elucidate whether modifications of this function could lead to pathologicalalterations of the thyroid gland as occurs in cholangiociliopathies or polycystic kidney disease (PKD).

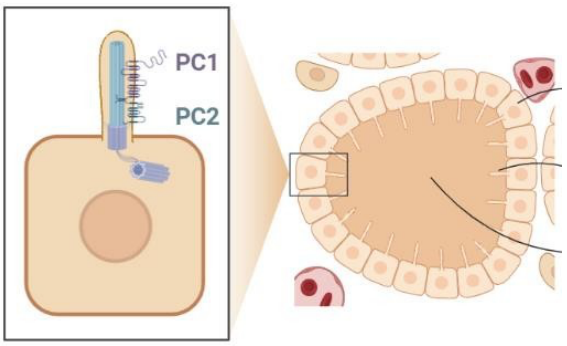

Figure 1. Localization of PC1 and PC2 in primarycilia of thyrocytes.

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C45 - Modeling Protein Dynamics in Age-related Macular Degeneration with Quantum Computing Algorithms

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Introduction: Quantum computing (QC) has the potential to transform the way Age-related Macular Degeneration (AMD) is understood, diagnosed, and treated, which could significantly improve the quality of life for individuals affected by this degenerative eye disease. In this regard, quantum computing can help better understand the complex molecular interactions involved in AMD, potentially leading to the development of more effective and specific treatments, delayingor even halting the progression of the disease. In the context of protein modeling related to AMD, it is crucial to know the three-dimensional structure of proteins to understand how they interact with other molecules in the eye and how they relate to disease progression. Based on previous studies regaring QC in biology applications [1, 2], it is used QC to more accurately simulate the geometries of molecular interactions that determine the three-dimensional structure of these proteins.
Method: A process with simplified algorithms is applied to simulate the structure of a model protein using a technique known as quantum sampling. A first modeling consists of two proteins: beta-amyloid protein [3] and the complement factor H (CFH) protein [4]. In the second simulation, quantum algorithms are used to simulate the molecular dynamics of proteins related to AMD. Thus, it simulates how proteins change shape and move over time, which can provide crucial information about their structure and function.
Results: The first simulation model represents a model protein using four qubits, where each qubit represents an atom in the protein. Quantum gates, such as the CNOT gate, are applied between neighboring qubits to simulate molecular interactions and the formation of chemical bonds. Subsequently, qubit measurement is performed to obtain information about the structureof the simulated protein. The second simulation initializes with a quantum register of 4 qubits to represent the initial structure of the protein. Then, quantum operations are applied to simulate molecular dynamics, such as rotating some atoms using rotation gates (e.g., Rz gates). Subsequently, qubits are measured to obtain information about the structure of the simulated protein.
Discussion & conclusions: Both models illustrate how QC can be used to simulate bothmolecular interactions and the temporal evolution of eye proteins related to AMD. It is importantto note that these are simplified examples and do not fully reflect the complexity in real proteins. However, they provide a basic introduction to how QC can simulate protein structures. In future applications, much more sophisticated algorithms and circuits will be used to accurately model AMD-related proteins and their molecular interactions.

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C46 - Histological study of the cornea of *Larus michahellis (Naumann, 1840)*

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Introduction: The cornea is a transparent protective refracting lens that contributes (up to two thirds)to the refractive power of the eye in air. Furthermore, mechanically it must be extremely tough to protect the inner contents of the eye. However, the corneas of birds, especially seabirds, have received little attention and studies of their functional morphology is limited. Using light microscopy and both scanning (SEM) and transmission electron microscopy (TEM), this is the first descriptionof the main components of the cornea in five individuals of the yellow-legged gull.
Methods: five adult yellow-legged gull were obtained from the Wildlife Recovery Centre in Santa Faz, Alicante. After dissection of eyeballs, macroscopic measurements were taken for axial and corneal length according to previous bibliography [1]. Subsequently, corneas were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned, and stained with Masson's trichrome and Miller's elastic fibers staining techniques. For the electron microscopy analysis, tissues were fixedin 2% glutaraldehyde and 4% paraformaldehyde, postfixed in 1% osmium tetroxide and embeddedin Epon812. Samples were gold sputter coated for SEM and analysed using a JEOL JEM-1400 Plus for TEM and a JEOL IT500HR/LA for SEM. Image analysis was performed using Fiji (ImageJ2), focusing on corneal layer thickness and keratocyte density. Statistical analysis was performed using GraphPad Prism version 9.
Results: Ocular parameters for *L. michahellis* were measured, revealing a mean eyeball diameter (T) of 22.24 ± 0.42 mm and a corneal diameter (C) of 12.03 ± 0.62 mm, with a C:T ratio of 0.54 ± 0.04, indicative of a spherical eye. Corneal thickness varied significantly between the central and peripheral regions (259.9 ± 29.39 µm at 292.6 ± 30.39 µm). Histologically, the cornea is composed of five distinct layers: the epithelium, Bowman's layer, stroma, Descemet's membrane, and the endothelium. The epithelium, a stratified flat type with 3 to 4 cell layers, transitions into Bowman's layer above a well-defined basal lamina. The stroma features a collagen-rich matrix interspersed with proteoglycans and elastin, providing structural integrity and optical clarity. Descemet's membrane supports the overlying endothelium, a single layer of flat cells with a cytoplasm with numerous vacuoles and tortuous basolateral membranes in intimate contact between adjacent cells. Many of these cells possess projections that rise above the membrane surface. The density of keratocytes was significantly higher in the central region (1605 ± 410.1 cells/mm²) compared tothe peripheral (630.7 ± 150.9 cells/mm²), indicating localized structural adaptations.
Discussion & Conclusions: The macroscopic parameters of the eyeball are similar to those of diurnal raptors. The cornea exhibits centro-peripheral differences in both epithelial and corneal thickness. The multilayered epithelium comprises columnar basal cells and flattened squamous cells near the surface, with apical cells covered by microvilli. Additionally, keratocyte density is higher in the central cornea than in the peripheral region. Ultrastructural analysis indicates that Bowman’s membrane is an irregular basement membrane displaying an irregular arrangement of collagen fibrils. No differences are observed in the structure or thickness of Descemet's membranebetween the central and peripheral areas of the cornea. The endothelium exhibits tortuous intercellular junctions and projections above the surface, manifesting as ruffled margins.

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C47 - Extracellular ATP balance in endometrial cancer varies with tumor grade

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Introduction: The levels of extracellular ATP and its derivatives such as adenosine are impaired inthe tissue microenvironment during tissue stress conditions such as hypoxia, infection, metabolic stress, tumor transformation and inflammation. Ectonucleotidases are the main regulators of extracellular ATP and adenosine levels, and their activity is altered in cancer. In the present work we have studied the ectonucleotidase expression in endometrial cancer by immunohistochemistryapproach.
Methods: Frozen paraformaldehyde-fixed tissue slides of 60 endometrial tumor samples have beenstudied. The analysis was carried out by two independent observers. The samples were observedand photographed and the distribution of the labeling was recorded and analyzed. The following information was recorded: absence or presence and label intensity in the tumor and stroma [null (0), mild (+), moderate (++) and high (+++)]. All data were recorded in tables. For statistical analysisthe absence or presence (and the level) of label, the grade and histological classification of the tumors were described using tables. Log-binomial models have been used to evaluate theassociation between protein and tumor type with 95% confidence intervals evaluated, whenever possible.
Results: Ectonucleotidases that are expressed in the epithelial component of the tumor in endometrial cancer are: NTPDase2, NTPDase3, NPP3 and CD73. According to this pattern, in tumor epithelial cells in endometrial cancer, the most efficient tandem in the hydrolysis of ATP to adenosine is NTPDase3-CD73. Stromal NTPDase2 expression correlates with the invasive capacity of tumors.
Conclusion: There is intra- and intertumoral heterogeneity in the expression of ectonucleotidases that correlates with tumor grade.

Acknowledgements: This work is supported by grant from the Instituto de Salud Carlos III (PI18/00541), co- funded by FEDER funds, and Asociación Española Contra el Cáncer (AECC).

Oral Presentations

Session of Friday 13th (11:00h -13:00h)

Oral 9 –Teaching Innovation in Histology

Moderators:
José Peña Amaro
Laura Robles Gómez

C48 - Hybrid adaptive flipped classroom methodology for teaching of Cell Biology in English in the Degree of Medicine of the University of theBasque Country (UPV/EHU)

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Introduction: University teaching in a foreign language is a challenge for both teachers and students who do not have it as their mother tongue. In the subject of Cell Biology, teaching in English has undeniable advantages, such as a high quantity and quality of teaching materials thatare often available online. However, teaching in English also requires the development of mechanisms to ensure a good understanding of the taught contents. The flipped classroom method is characterized by students working on the theory contents before they are taught in class. One of the main advantages of the flipped classroom compared to traditional lectures is that it makes it easier to move from a purely descriptive approach to a more in-depth and interacting approach, facilitating the generation of face to face debates and critical discussions with students [1]. However, the weak point of this method is that it also requires a high level of involvement and responsibility on the part of the students themselves.

Methods: We report the experience of three consecutive academic years (2021-2022, 2022-2023,and 2023-2024) applying a hybrid adaptive flipped classroom methodology for theory lectures of Cell Biology, a subject belonging to the first year of the Bachelor's Degree in Medicine at the University of the Basque Country/Euskal Herriko Unibertsitatea (UPV/EHU). The innovation sought to encourage student participation in the lectures, as well as to improve understanding of critical concepts. A total of 142 students took hybrid flipped lectures, and 95 of them (years 2022- 2023 and 2023-2024) responded to questions related to this methodology. Statistical analysis was performed by IBM SPSS Statistics v.28.1.

Results: The hybrid flipped classroom methodology was effective in promoting student participation in Cell Biology theory lectures. A majority of students declared that it took them an average of 30min-1h to prepare for each lecture. Students preferred this dynamic tothe traditional lecturing model (Figure 1), highlighting the possibility of getting bonuses for participation. However, stark differences were observed among students with regard totheir overall preferences of classroom attendance, not only in Cell Biology but alsoin other subjects of the degree. Interestingly, about one quarter of respondents declaredthat enrolling in Cell Biology helped them improving their general level of English (Figure 1), and this perception was greater in students that possessed a lower initial knowledge ofEnglish (B2) before taking the subject (Fisher's exact test; $p<0.001$). The overall good perception on the part of the students was underpinned by some very positive comments to open questions about what they would highlight about taking Cell Biology in English. Some of them also declared that the flipped method was useful to help them prepare better the exams.

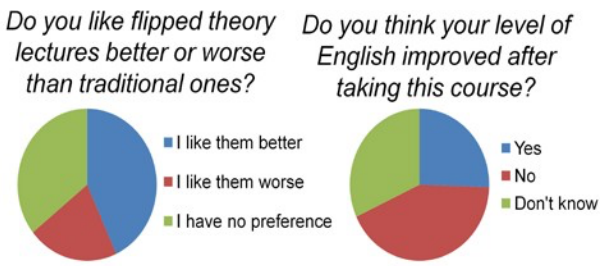

Figure 1. Students' responses to some questions related to flipped theory lectures in Cell Biology

Discussion & conclusions: The experience of three consecutive years (2021-2022 onwards) applying a hybrid flipped classroom methodology was positive for the teacher and also for an important part of the students. Students' participation and attendance to lectures were improved. No upward or downward changes in academic grades were observed in the last three years, despite the fact that several students declared that the flipped lecture method facilitated their learning and exam preparation. Students with a lower initial level of English (B2) reported a greater improvement in their overall level of English after taking this course.

[1] Prieto-Martín A, et al. (2019). Aula invertida en enseñanzas sanitarias: Recomendaciones para supuesta en práctica. Revista Fundación Educación Médica (FEM). 22 (6), 252-262.

C49 - Effectiveness of peer assessment using the Moodle workshop activity

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Introduction: Peer assessment is an educational strategy that tries to achieve a double objective: to evaluate students' learning and to offer them a second learning opportunity by evaluating classmates' exercises. When these exercises are done in the classroom, the time available limits that each student can evaluate one or two works of their peers, but Moodle offers an activity (WORKSHOP) that simplifies this type of tasks because [1]:

- It allows the activity (completion of the exercise and correction) to be carried out in the classroomor in a remote way.
- The non-presential correction allows each student to evaluate several assignments.
- Students evaluate their classmates using a rubric provided by the teacher in Moodle.
- After the evaluation, Moodle gives each student a mark with the average of all the scores givento that student by classmates.
- The calculations are automatic, which allows the tool to be used with a large number of students.
- The teacher's activity consists of:

- Give the students the task information and an evaluation rubric.
- Reviewing the evaluations made by the students and adjusting them when they are not correct.

Methods: We are using this tool in the first year of dentistry at the School of Medicine and Nursingof the UPV/EHU. We have carried out eight workshops. In four of them, the students had to answerwritten questions. In three of them, they had to make drawings or diagrams of histological structures, and in the last workshop they had to give a name to some structures indicated in a drawing.

Results: We have analysed the difference between the marks given by the students and those estimated by the teacher, and we have observed that this difference may vary in each workshop (Figure 1). A provisional analysis indicates that the difference depends quite a loton the type of workshop carried out, since in the workshops where the students had to make drawings, the differences in the grades were greater than in the workshops where they had to make a written answer. The workshop in which they had to give names to the structures marked in a drawing showed the smallest differences.

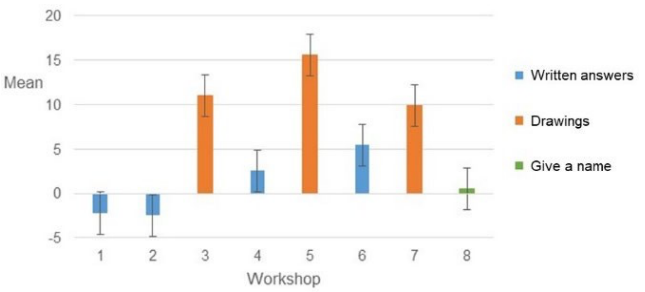

Figure 1. Mean of the differences between the marks assigned by the students and the one given by the teacher in the eight workshops. Each type of workshop is indicated by

Discussion & conclusions: Our perception is that the type of workshop conducted implies an evaluation rubric with certain characteristics, and that this affects the effectiveness of the peer assessment. Probably, the higher differences found in the drawing exercises indicate that it is morecomplicated to elaborate a good evaluation rubric.

[1] García González D. (2020). Teach Twice: How to set up MOODLE workshops to get your studentsassessed by each other. Triple Eñe, Bilbao, Spain.

C50 - A new teaching-learning resource in the observation of histological slides under the light microscope: interactive images

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Histology subjects of the Degree in Medicine are indispensable for the acquisition of basic conceptsfundamental for the subsequent clinical courses. In addition to the theoretical classes, in the practical training several histological preparations are observed. The usual methodology involves a brief explanation of the tissue to be studied by the teacher and students work individually on thesespecimens under their light microscope. Many of them choose to take pictures with their own cell phones, through the eyepiece, to have a repository of images for future study (of low quality due tothe rudimentary technique). At the end of the semester, the students must pass a practical test based on the observation of images of different light microscopy slides. The academic results of these exams are almost always worse than those obtained in the tests based on theoretical contents. In addition, as a continuous evaluation activity, the students must prepare a practice notebook with drawings and explanations of the histological preparations studied in practice. The correction of these notebooks shows that some students do not assimilate the concepts well and make mistakes. In order to try to solve these academic deficiencies, the use of other innovative teaching methodologies that may help students to acquire competencies is essential.

In this teaching innovation project, high-resolution photographs of histological slides will be taken with professional equipment specially prepared to obtain photographs using light microscopes. These photographs will be worked with Genially, an online tool for creating interactive and animatedcontent, to obtain interactive images that will include labels with the different structures of the preparation, windows with magnified images of the tissue for better observation and a collection of review questions, among other contents (See Figure 1). These interactive images will be included in QR codes that will be attached to the slide of the preparation. Students will be able to scan the code, so theywill always have this material available to review and understand what has been studied. In this manner, it will allow the student to use all the practice time for detailed observation of the preparation, without wasting time trying to take pictures with their own cell phones. The students will always have this study tool available for free, at any time and place, aslong as they have internet access. Once the microscopic observation practices have been completed, students will answer a satisfaction survey on the Moodle platform so that they can anonymously evaluate the usefulnessof the interactive images. For this purpose, the survey will consist of a series of questions with response options on a scale of 0 to 5 (0 being very dissatisfied and 5 very satisfied). In addition, the survey will contain a final section for comments, where the students will be able to expose themost outstanding aspects of the new tool and aspects of improvement for the future. Based on theresults, the suitability of the innovation proposal can be evaluated. By analyzing the data obtainedand the academical results of the practical part, it will be possible to design strategies for improvement, expansion of content and correction of possible weaknesses in the project.

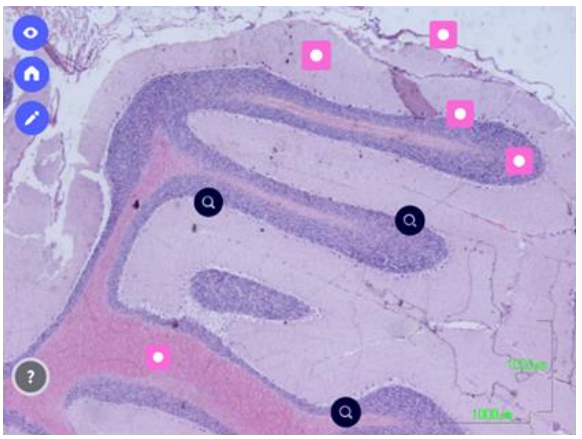

Figure 1. Example of a histological image with several interactive buttons made with Genially

C51 - Continuous evaluation in the subject of Pathology. Proposal for teaching improvement from concepts based on Histology.

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Introduction: Pathology is a discipline that provides the basis for understanding disease in medicinethrough morphology. In recent years, the aging of the teaching staff and study plans that are not close to the reality of PA in attendance have worsened the situation. Student opinions and thoughtsare central to the question of whether and how such curricula should be modernized. The objectiveof the study was to know their previously acquired knowledge in the field of Histology, as well as toverify their academic results in a continuous evaluation program.
Methods: A survey was carried out on 148 Medicine Degree students at a Faculty in Andalusia about their preferences in pathology teaching modalities and their satisfaction with face-to-face courses, as well as a continuous evaluation of the knowledge acquired in the theoretical class andreinforced in the practical classes, taking into account the basis of their concepts in Histology. A qualitative analysis was carried out comparing the responses obtained and the differences between practice and theory.
Results: Student satisfaction with the lecture-based curriculum was positively correlated withstudent grades (Spearman correlation coefficient 0.21). Furthermore, students with lower gradessupported the continuous model prevailing (Spearman correlation coefficient 0.41). The majority supported virtual microscopy, autopsies, seminars, and podcasts as preferred teaching methods.
Discussion & conclusions: The data supports the implementation of an AP curriculum wheretutoring, post-mortems, and supplemental learning tools play an important role. As well as a trueapproach of the subject to the reality of the specialty, always based on constructive knowledge andsupported from normality to pathology.

[1] Hassell, L. A., Absar, S. F., Chauhan, C *et al*. (2023). Pathology Education Powered by Virtual and Digital Transformation: Now and the Future. *Archives of pathology & laboratory medicine*, *147*(4), 474–491. https://doi.org/10.5858/arpa.2021-0473-RA
[2] Bashir, A., Wilkins, K., & Pallett, R. (2023). An Innovative Workshop Embedding Pathology Service Users into the Undergraduate Biomedical Science Curriculum. *British journal of biomedical science*, *80*, 11584. https://doi.org/10.3389/bjbs.2023.11584

C52 - Collaborative peer-review for first-year medical students: a pilot experience

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Introduction: Peer review has been shown to offer many benefits for undergraduate medical education, for instance, students can evaluate their knowledge and critical skills. Additionally, team-based learning helps them to interact and develop professional behavior. The present work aims to evaluate the advantages of combining both teaching strategies. This pilot experience was developed during the first course of medical degree from the University of Malaga in the frameworkof the practical curriculum of the subject Cytology, Genetic and Human Development. Methods: The subject was taught during the first semester of the 2023/2024 academic course.175first-year medical students were assigned in 44 groups of 4 students during practical classes. The groups elaborated two separate reports about specific laboratory practices selected from the curriculum: mitosis and gametogenesis. Reports should include microphotographs taken from microscopic and/or virtual slides analyzed during practical classes and were written according to templates provided by the teaching staff. In every single group, one student assumed the role of the corresponding author. Reports were submitted and randomly assigned to peer-review groups using the tool *workshop* from Moodle virtual environment. Groups received detailed instructions forconducting the evaluation and providing appropriate peer feedback together with an evaluation rubric. Finally, original reports and co-evaluations were evaluated, corrected (when necessary) andresubmitted to the platform by the teachers, assuring a constant feedback loop and the transparency of the process. To evaluate the impact of this activity on the student-body a closed questionnaire with answers using a 5-point Likert scale survey was used. To capture other dimensions about student perceptions, a free-text field was included at the end of the questionnaire.Results: Most of surveyed students (78%) reported more involvement during these two practices than in the others from the same subject. They also indicated having more interaction with their peers in the laboratory, and as a consequence of elaborating these reports as well (80%). Moreover, students agree that this activity helped them to improve not only their knowledge aboutthe contents (74%), but also their writing and collaborative skills related to reporting exercises (70%). Importantly, 50% students acknowledge having identified some mistakes and weaknessesof their own works during the co-evaluation or having learnt from their peers' reports. Nevertheless, even if the marks awarded by students and teachers did not diverged substantially from a quantitative point of view, the qualitative contents were not always well assessed by peers. For thisreason, the final feedback from faculties was highly regarded by the student-body. On the other hand, 60% considered they have not yet understood enough the scientific publication system. In fact, students requested more information about evaluation and peer-review process to better perform this task and to be aware of how scientific editorials and journals work. Overall, 60% of students affirm to have enjoyed the activity (4-5 according to Likert-scale). Discussion & conclusions: Early-stage medical students found this first contact with group reportingand peer-review process quite useful and innovative. They showed a higher level of engagement during these two specific practical classes and enjoyed the team-based learning in the laboratory.Moreover, students perceived they were working not only specific but also general competencies.For a comprehensive experience, students have demanded more detailed information about the peer-review process before starting the first co-evaluation round. In addition, the current evaluationrubric needs amendments so reviewers can provide a highly accurate peer feedback. Finally, peer-review in a collaborative learning environment may aid for a sound education, professional trainingand the acquisition of behavioral skills.

C53 - Students' perceptions on Virtual Microscopy in Medicine Education

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Introduction: The use of virtual microscopy (VM) has already been demonstrated to enhance student learning and overall performance in a dynamic learning environment, and it is highly accepted and adopted by several medical schools across the globe. Several Medical Programs have adopted the VM to complement the effectiveness of competency-based education in medicaleducation. The major challenges in implementing VM platforms are the need for a complete set ofslides to cover all the topics on the medical curricula and the cost of developing a readable and functional interface. To solve it, we joined a European consortium through the Erasmus+ program "KA220-HED-Cooperation partnerships in higher education", with the participation of partners fromRomania, Bulgaria, Poland and Greece. The project granted, entitled "Digital Transformation of Histology and Histopathology by Virtual Microscopy (VM) for an Innovative Medical School Curriculum", is currently under development and will be resumed by the end of this year. Betweenthe project's objectives, we wanted to evaluate the acceptance and opinions of our students aboutthe introduction of VM technology in their classes.

Methods: We actively engaged our first-year students of Medicine degree in the research processby asking them to fill out a survey with 10 sentences marked on a 5-level Likert scale (from totally agree (TA) to totally disagree (TD)). Precautions were taken to avoid automatized answers, like changes in the positive-negative order of the possible answers.

Results: From the total number of students enrolled on General Histology (85), we obtained answers from 39 of them. All the students affirmed that the use of VM has significantly helped themto reinforce the contents studied and seems to be a positive tool, with a 77% TA for both sentencesand no negative answers. The sentence with more TA answers was related to the quality of the images in VM, being perfect for learning purposes for 90% of the students. On the contrary, only 5% of the students showed some sort of difficulty using VM. 60% of the students consider that VMhas reduced the time needed for them to learn histology sections. Finally, only one of the studentssurveyed was in favour of substituting all the laboratory practices on observation of histological slides through the microscope with VM contents, while 90% would like to maintain a mixture of laboratory and VM sessions.

Discussion & conclusions: By analysing the survey answers, we can affirm that our students thinkthat VM is a helpful tool to improve their learning of General Histology. Moreover, the current technological development of the VM has achieved a high-quality standard of images, sufficient to accomplish the necessary competencies. We can also assume that our system is easy to use anddoes not represent a difficulty in the learning process. While traditional laboratory practices are thepreference of our students, the introduction of VM sessions is a positive addition for them. These results encourage us to continue developing and introducing VM in the learning-teaching process of Histology.

Supported by 2022-1-RO01-KA220-HED-000089017 Erasmus+ project from the European Commission

C54 - Histology to Health Sciences Students: Use of Telegram® as a complementary tool for active and collaborative learning

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Introduction: Histology is a 1st-year basic training subject in the degrees in Physiotherapy and Nursing of the Faculty of Health Sciences (FHSS) at the University of Valladolid (UVa). Online teaching, linked to the use of communication and information technologies, is an innovative teaching model because it breaks the time and space gaps that face-to-face education entails. Thestudents become protagonists and builders of their learning, and the teachers become, mainly, tutors and counselors. This study aimed to evaluate the use of Telegram® in the Histology subject in 1st year Nursing and Physiotherapy students at the UVa FHSS during the 2023-24 academic year. Methods: At the beginning of the course, a Telegram® channel was created to share contentand images, ask questions, discuss the identification of histological structures or cell types, etc. Periodically, the teaching faculty asked questions about the contents and asked the students to participate in analyzing different histological images. At the end of the semester, the faculty developed, on the channel itself, a questionnaire to collect the perceptions of the following dimensions: connectivity, benefits, usefulness, and learning, from the students about the designedtool. Results: The sample consisted of 117 students (18.3±1.2 years old; 72 women & 45 men, 53Physiotherapy & 64 Nursing). 100% of the students declared using Telegram® for educational purposes for the first time, and channel was easy. Of the benefits of Telegram® 45.6% reported that it was easy to use and 24.9% that it made it easy to stay up to date with news. Regarding usefulness, 31.3% and 24.8% reportedan improvement in student-teacher communication and the ability to offer new learning respectively, although 5.2% declared that it was not useful. 94.3% would apply Telegram® to improve their academic results, 45.6% would recommend it for future courses in the subject of Histology and 36.1% in other subjects of their degree (Table 1). Discussion & conclusions: Thehigh number of passes in Histology and the students' recommendations for future courses supportthat Telegram® be integrated into the teaching-learning process considering the characteristics of the students as a complementary tool in the theoretical and practical Histology lessons.

Table 1. Results of the Teaching Innovation Project Use of Telegram® as a learning tool in Histology. The results of the categorical variables are expressedas a percentage (frequency)

Telegram® benefits			**Telegram® utility**		
Provides greater course communication		18.2 (21)	Increases attention in class		2.5 (3)
It's easy to use		45.6 (53)	Improves student-teacher communication		31.3 (37)
Makes it easy to stay up to date on news		24.9 (29)	Offers new learning		24.8 (29)
Provides news information		11.3 (14)	Offers a new way of communication between colleagues		16.7 (20)
Telegram® application			Promotes greater participation		19.5 (23)
Learning motivation		3.1 (4)	It is of no use		5.2 (5)
Improves academic results		94.3 (110)	**Future of Telegram®**		
Feedback on own learning		2.6 (3)	Would you recommend it for other subjects for the degree		36.1 (42)
Telegram® Connectivity			Would you recommend it in future Histology courses?		45.6 (54)
1st time I used Telegram® Teaching		100 (117)	I would not recommend it in any case		18.4 (21)
Mobile phone operating system	Android	74.3 (87)	**Academic Results**		
	iOS	25.7 (30)	Theoric exam	Failed (F)	6.0 (7)
				Approved (C)	63.2 (74)
				Remarkable (Notable??) (B)	19.6 (23)
				Outstanding (A)	11.2 (13)
Internet connection type	5G data	98.3 (115)	Practical test	Failed (F)	1.7 (2)
				Approved (C)	37.9 (44)
				Remarkable (Notable??) (B)	56.1 (66)
	Wifi	1.7 (2)		Outstanding (A)	4.3 (5)
Installation of the Telegram® Channel"UVa Histology"	Easy	83.7 (98)			
	Regular	12.8 (15)			
	Difficult	3.5 (4)			

C55 - 'Loquent images': recreation of histological tissues through their etymological roots.

Noguera Salvá R.[1,2], Martín de Llano J.J.[1], Ruiz Sauri A.[1], Monferrer Garzarán E[3], Cantillo Lucuara, M.[4], Navarro Noguera A.[5] and Movellán Luis M.[6]

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Introduction: The Consolidated Teaching Innovation Group at Valencia University, FILOMED: Histological keys between classics and moderns (GCID23_2588748) presents on the web https://histetim.blogs.uv.es/ the results of the various innovation projects it has developed in recentyears. 1) Histology in vignettes and its Greco-Latin etymological correlate (2021-2022). 2) Histology and Philology: is a picture worth a thousand words? (2022-2023). 3) Loquent images: recreation of histological tissues through their etymological roots (2023-2024). Our projects represents an interdisciplinary endeavor, aiming to integrate Humanities with Health Sciences to facilitate the study and retention of medical terminology. Notably, the pedagogical approach towards teaching and memorizing technical lexicon within the medical domain has been extensively discussed in scholarly literature. Empirical findings commonly indicate enhanced learning outcomes when employing an etymological perspective, particularly rooted in Greco-Latin origins. This methodology facilitates the establishment of semantic connections between terms and offers mnemonic aids, thereby bolstering the acquisition and retention of medical vocabulary [1].

Methods: Our objective is to harmonize the fields of Medicine and Philology through the utilizationof comic imagery to augment students' lateral thinking and autonomy [2]. Our approach involves the development of histological-etymological worksheets, wherein we furnish students with a comprehensive array of scientific-medical terms pertinent to histological specimens of diverse human tissues, accompanied by original graphical representations derived from caricaturizing (see Figure 1 and https://histetim.blogs.uv.es/materiales-docentes/). The overarching goal is to furnish students with a more profound methodological framework to enhanced comprehension and retention of subject matter.

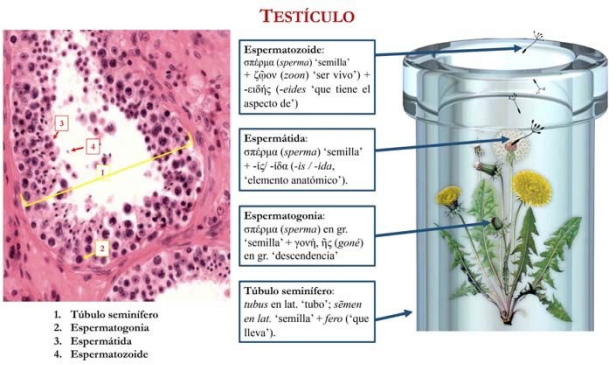

Figure 1. Example of an etymological worksheet.

Results: The assessment of students' self- directed learning, facilitated through Moodle questionnaires, reveals encouraging outcomes. Additionally, final evaluations indicate a positive correlation between the utilization of worksheets and the incorporationof etymology into the curriculum, suggesting abeneficial impact on the retention of vocabulary among students. Regarding open science practices, the establishment of the website signifies an open dissemination of the developed materials. In its inaugural year (April 2023-April 2024), the website has garnered substantial traction, amassing approximately 3500 visits primarily from Spain and Latin America.

*Project granted by the Vicerrectorado de Transformación Docente y Formación Permanente de la Universidad de Valencia (UV-SFPIE_PID-2732134).

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C56 - Integration, assimilation and relevance of Histology in Pharmacy and Human Nutrition and Dietetics students at the UPV/EHU

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Introduction: A good conceptual basis of the histology of organs and systems seems essential to develop a good praxis in health professionals. The UPV/EHU offers the degrees of Pharmacy andHuman Nutrition and Dietetics, as well as the double degree, in the Faculty of Pharmacy. The subject Cell and Tissue Biology (6ECTS) enables students to acquire competences related to the understanding of the organization at the cellular and tissue level of the human body. In the third course of the Pharmacy Degree, General Structural Biopathology (optional subject with 6ECTS) provides knowledge of molecular, cellular and histological changes in the pathological condition. Our hypothesis is that the histological training of first-year students is insufficient for future pharmacists, dietitians and nutritionists to understand the importance of tissue organization, considering that their professional practice will affect organs and, consequently, tissues and cells.With these premises, the aim of the study is to evaluate the extent of the knowledge of histology, throughout the degree, of pharmacy and human nutrition and dietetics students, as well as their perception of the importance of this knowledge in their future practice as health professionals.
Methods: The procedure consisted of a conceptual multiple-choice test to assess the knowledge of histology (of 2nd and 4th year students) who had already passed the subject of Cell and TissueBiology. The results were analyzed based on the previous scores obtained in the assessment of this subject. The students also rated their subjective perception of the importance of the subject fortheir future professional practice from 1 (not at all) to 4 (very much).
Results and Discussion: Analysis of the results obtained in the conceptual test showed that basic knowledge of histology had been maintained, since the average mark of the study population wasa pass. The trend in both degrees was similar, so that the grades obtained in the study were lowerthan those obtained in Cell and Tissue Biology. This may be due to the loss of some of the knowledge acquired. In all cases, students in the 2nd year obtained higher average marks than those in the 4th year. When comparing between degrees, we observed that the average marks aresomewhat lower in Human Nutrition and Dietetics students than in Pharmacy students, but similarto what occurred in the marks of the subject in the first year, and their ratings decreased by 8.6% and 11.9%, respectively. When comparing the population of 4th year students who had studied General Structural Biopathology with those who had not, we observed a very significant improvement in the mean marks obtained in the study. The students' subjective perception of theirknowledge of histology was, for the most part (65%), sceptical, as they felt that they would have problems identifying the different tissues with certainty when viewing a sample under the light microscope, and only a very small proportion (5%) felt comfortable identifying tissues with absolutecertainty under the microscope. Furthermore, in the responses obtained on how they perceive theirknowledge of histology for practicing as health professionals, slightly more than half of the samplesurveyed (54%) considered that their knowledge of histology would be sufficient when it came to developing their professional practice. A considerable proportion (42%) of the sample of students who had studied General Structural Biopathology felt that subject had helped them to consolidate the histological knowledge they had obtained in the main subject and feel more confident in the knowledge they possess, a fact that will help them to develop their professional practice.
Conclusions: Our findings reported that despite a slight decrease in the knowledge acquired in bothdegrees, the students maintain a similar level of knowledge of histology to that obtained at the endof the subject Cell and Tissue Biology. Students who have also done the subject General StructuralBiopathology improve their level of knowledge of histology, and also consider that they have greaterskills and feel more confident about their knowledge of histology, which will make them feel more comfortable in their future professional practice.
This research was funded by the Basque Government grant number IT1751-22.

Poster Presentations

Poster Presentations

Session 1

Histopathology
Neurohistology

Histology and Histopathology
From Cell Biology to Tissue Engineering

P01 - Ultrastructural study of rabbit VX2 liver tumor: metastasis and necrosis

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Introduction: Since its introduction over 70 years ago, the rabbit VX2 carcinoma has been extensively used as a model of liver tumors to assess various treatment modalities that require animals larger than rodents. The VX2 tumor is a squamous cell carcinoma derived from *Shope papillomavirus* infection in rabbits, characterized by rapid growth. Its implantation techniques or growth characteristics have been accurately described, but information about its biology is scattered, to date, no ultrastructural study of the model has been carried out. The objective of this work is perform an analysis of the biological processes that lead to metastatic development and tumor necrosis.

Methods: To follow the development of the tumor we have carried out a tumor implant in 8 adult New Zealand white rabbits (weight: 2.5–3.0 kg, male) which were sacrificed in pairs at 4, 8, 11 and 17 days after implantation. Fresh tumor tissues were obtained from VX2 tumor-bearing rabbits that were subcultured in our laboratory. Briefly, fresh tumors were cut into small pieces of 1 mm3 under aseptic conditions, then a piece of tumor was implanted orthotopically into the middle liver lobe adjacent to the gall. The tumors were dissected within the established time and the biopsies were processed to perform histological and electron microscopy techniques according to conventional protocols. All the animals were used under approved animal care and using committee protocols.

Results: After 8 days of growth tumors are well delineated and composed of lobules of large undifferentiated cells with a high nucleo- cytoplasmic ratio. Their nuclei are large, round, and with prominent nucleolus that, due to its high activity in the synthesis of ribosomal particles, sometimes extends to the nuclear envelope. In the cytoplasm of tumor cells, few granular endoplasmic reticulum saccules, few mitochondria and a large number of free polyribosomes are observed

(Fig.1). The cells are closely linked to each other by desmosomes characterized by their large bundles of tonofilaments. The different lobules of tumor cells appear covered by a narrow fibrous layer formed mainly by fibroblasts. After 11 days of tumor growth, we can observe the ultrastructural characteristics of the epithelial-mesenchymal transition (EMT) process, associated with the first steps of the metastasis. Tumor cells must break loose and acquire a mesenchymal phenotype in order to migrate. The rupture of the desmosomes is visualized by the separation of the cell membranes, and the appearance in their cytoplasm of the detached tonofilaments. The presence of autophagic vacuoles is also notable. From the periphery of the tumor, the cells are released, gradually acquiring a mesenchymal phenotype that allows them to move. Some of them present primary cilium. Intermediate phenotypes are also observed. From 14 days the largest tumor sheets or lobules are generally centered by necrotic areas containing necrotic and apoptotic-cell debris, and few inflammatory cells, mostly macrophages and lymphocytes. A cytoplasmic vacuolization begins to be observed that corresponds to a mitochondrial alteration, due to lack of oxygen, in which the rupture of the cristae and even the mitochondrial membranes occur.

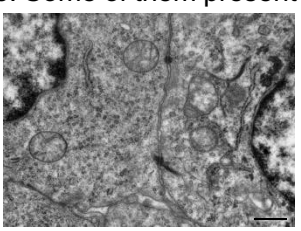

Fig.1. Ultrastructural characteristics of tumor

Discussion & Conclusions: Our findings establish two temporal parameters, metastasis and necrosis, that affect tumor survival. These data will serve to guide the design of future preclinical research using this model.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P02 - Cell communication mechanism based on spheresomes in low and high-grade brain tumours: an ultrastructural study.

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Introduction: Cells communicate with each other through extracellular vesicles (EVs), including exosomes or ectosomes (also known as microvesicles). The heterogeneous EVs population consists of several subpopulations with different biogenesis, which implies variations in size, in morphology and in composition. These cell-derived membranous structures transfer different biomolecules and play a role in various physiological and pathological processes. In 2016, spheresomes were described as a new type of EVs by Junquera *et al.*[1] in gastrointestinal stromal tumours. These EVs originate from vesicles derived from the reticulum (RER)-Golgi pathway and aggregate beneath the cell membrane. As the vesicle count rises, the membrane protrudes into the extracellular space, forming a multivesicular sphere (MVS) which is finally released. The rupture of the MVS membrane enables the release of spheresomes into the extracellular medium near other cells. Methods: A total of 5 low-grade gliomas and 4 glioblastomas from patients attended in the Neurosurgery Department from the Hospital Universitario Lozano Blesa (Zaragoza, Spain) were included in this study. The samples were routinely processed to study them using a Transmission Electron Microscope (TEM). The three-dimensional (3D) reconstructions were performed from 8-12 serial ultrathin slices by manual segmentation of the images using Image J Fiji software (National Institutes of Health, Bethesda, MD, USA, v1.53). All procedures were approved by the Human Research Ethics Committee (Comité Ético de Investigación Clínica de Aragón, CEICA) from the Instituto Aragonés de Ciencias de la Salud (IACS) (permit numbers: PI16/0324, PI17/255). Results: In low and high-grade gliomas, only spheresomes and exosomes were found in ultrastructural studies. Morphologically, spheresomes and MVS (Fig. 1) are both larger than exosomes and multivesicular bodies (MVB). MVS (harboring spheresomes) are 3.5-4 times more frequent than MVB (with exosomes) in both gliomas and glioblastomas. In fact, no free exosomes were found in the extracellular space, while MVS with spheresomes inside were frequent. Therefore, cell communication through EVs occurs mainly through spheresomes in these tumours. Spheresome-laden MVS do not only appear in the extracellular space, but also inside blood vessels. Therefore, these EVs are able to cross the blood-brain barrier to reach distant tissues. In addition to describing the biogenesis and transport of these EVs in low and high-grade gliomas, in this communication we show for the first time the 3D reconstruction of these structures. Discussion & Conclusions: Exosomes, ectosomes and spheresomes share lipid bilayer, morphology and size, which complicates their microscopic and biological differentiation. So far, the most studied EVs are exosomes and ectosomes [2]. However, because of their predominance, more attention should be paid to spheresomes. Since the distinct biogenesis processes of EVs result in diverse cargo profiles and varied functional roles, deeper studies on EVs will contribute to our understanding of glioblastoma progression and metastasis, shedding light on potential avenues for targeted therapeutic strategies.

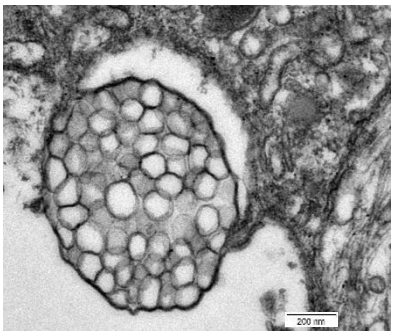

Fig.1. MVS with spheresomes.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P04 - Histopathological analysis of growth of implanted VX2 liver tumor

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The rabbit VX2 model of cancer has been widely used to demonstrate the efficacy of treatments against liver tumors. It is an anaplastic squamous cell carcinoma derived from Shope papillomavirus infection in rabbit. The tumor can be serially transplanted from one animal to another by allograft implantation and may grow in almost any grafted organ. Although there are many articles related to the VX2 model, basic research articles are very few and give a sparse description of the tumor [1]. The aim of this study was to explore the tumor characteristics focus on the early stages of growth.

A total of 12 New Zealand white rabbits were used in the present study. In each animal, 1 mm^3 of thawed VX2 tumor was implanted into the middle lobe of the liver. Two rabbits were sacrificed at 2, 4, 8, 11 ,14 and 17 days after implantation and the implanted lobes were collected and fixed in 10% formalin. Procedures were approved by the Animal Experimentation Ethical Commission, University of Zaragoza (permit number: PI35/22). Formalin-fixed tissues were trimmed, the volume of the tumors was determined and samples were processed according to standard histopathological procedures. In addition, tissue sections were stained with Masson`s trichrome stain, and processed for immunohistochemistry (IHC) using the following primary mouse monoclonal antibodies: anti-cytokeratin 7 (CK7), anti-actin (SMA), anti-vimentin and anti-p53. All antibodies were obtained from Dako/Agilent (CA, United States).

Macroscopically, a well-demarcated white lesion was observed under the hepatic capsule. Lesion enlarged rapidly from 6 mm^3 at day 2 to 400-600 mm^3 at day 17. On days 2 and 4, the lesion was characterized histologically by the presence of the implanted tissue composed of necrotic cellular debris and residual connective septa. IHC for p53 and CK7 revealed scattered foci of tumor cells in the periphery of the implanted tissue. The lesion was outlined by a thin rim of vimentin-positive cells which included activated hepatic stellate cells (SMA-positive cells). On Masson`s trichrome stain sections, there was no staining of the collagen at the interface between the liver and implanted tissue. On day 8, the lesion was characterized by the presence of residual implanted tissue surrounded by a thick ring of high density of vimentin-positive cells. The ring of cells was composed of thin sheets of tumor cells surrounded by large fibrous septa containing SMA-positive cells and dense immune infiltration. A fibrous capsule located between the cellular rim and liver parenchyma was observed in both cases. From day 11, the following events were observed: i) the implanted residual tissue progressively decreased; ii) spontaneous necrosis was present and increased with tumor size forming cystic cavities; iii) the fibrous septa and the immune infiltration into the tumor decreased drastically; iv) a fibrous capsule with variable immune infiltration was present in all cases.

Information on implantation techniques and VX2 tumor growth is abundant, but to our knowledge, histopathological characteristics at very early stage after its implantation have not been previously reported. The current study may lead to a more complete understanding of tumor biology, which is essential for the design of experimental studies.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P05 - Expression of PDL-1 and I in oral squamous cell carcinoma and its relationship with local and distance recurren

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Squamous cell carcinoma of the oral cavity (OSCC) is a carcinoma with squamous differentiation that arises of the oral mucosa epithelium. The oral cavity includes the mucosal surface of the lips, tongue, floor of the mouth, the buccal mucosa, the retromolar trigone, the maxillary and mandibular gingiva and the hard palate. CCEO is a malignant neoplasm most common in the oral cavity and represents more than 90% of its malignant neoplasms. It is the 16th most common cancer common in the world with an incidence of 377,713 new cases in 2020 and the 16th cancer in mortality of all types of cancer, with a mortality close to 50% at five years. It is more common in Asia and Europe and, Among its risk factors are tobacco and alcohol, which have a synergistic action, and infection with the HIV virus. human papilloma. Other risk factors are betel quid, chewing gum, Epstein-Barr virus, exposure to ultraviolet rays and diet. In recent years there have been variations in the geographical distribution and in the traditional risk factors with the emergence of new risk factors, which is exerting a modification in the approach of prevention, diagnosis and treatment. This is intended to try to improve the prognosis, which carries a high overall risk of death and significant morbidity. cell carcinoma Squamous cell carcinoma represents a serious and dynamic global health problem that requires study. In this sense, we have identified how there is a significant variation in the expression of PDL-1 and I in our cohort in relation to local and distant recurrence.

Keywords: Biomarkers; histological markers; oral cavity; squamous cell carcinoma.

Histology and Histopathology
From Cell Biology to Tissue Engineering

P06 - Study of placental tissue calcification and its molecular pathways in hypertensive states during pregnancy

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Pre-eclampsia (PE) is a complex multisystem disease characterized by sudden-onset hypertension (>20 weeks of gestation) and coupled with the presence of at least one additional complication, such as proteinuria, maternal organ dysfunction or uteroplacental dysfunction. Hypertensive states during pregnancy carry life-threatening risks both for the mother, including reduced life expectancy, increased risks of stroke, cardiovascular disease and diabetes, and the baby, such as increased risks of preterm birth, perinatal death and neurodevelopmental disability and cardiovascular and metabolic disease later in life. The pathogenesis of PE develops due to a dysfunctional placenta with aberrant architecture that releases factors contributing to endothelial dysfunction, an antiangiogenic state, increased oxidative stress and maternal inflammatory responses. Previous studies have highlighted a correlation between grade 3 placental calcifications and an elevated risk of developing PE at term. However, little is known about the molecular pathways leading to placental calcifications. In this work, we have studied the gene and protein expression of c-Jun N- terminal kinase (JNK), Runx2, osteocalcin (OSC), osteopontin (OSP), pigment epithelium-derived factor (PEDF), MSX-2/Hox8, SOX-9, Wnt-1 and β-catenin in the placental tissue of women with PE. Additionally, we employed von Kossa staining for detecting mineral deposits in placental tissues. Our results show a marked increase in all these components in the placentas of women who have undergone PE. Therefore, our study suggests that PE and the imbalance of the factors released may be associated with the activation of the molecular pathways of placental calcification. This knowledge is the starting point for future research aimed at describing the molecular mechanisms that promote placental calcification in EP, and the development of therapeutic strategies directed against it.

Keywords: Placenta, pre-eclampsia, calcification, JNK, MSX-2/Hox8, Wnt-1

Histology and Histopathology
From Cell Biology to Tissue Engineering

P07 - Identification of new tissue markers for the monitoring and standardization of penile cancer

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Penile carcinoma is a rare urological neoplasia that has a prevalence of 0.58-1.33 per 100,000 men in developed countries, it differs with countries that have low and middle-income where they have more incidences. In addition to age and ethnicity, the main risk factors include smoking, poor hygiene, and phimosis. It is estimated that up to 20-50% of cases of penile cancer are associated with infections caused by the human papillomavirus (HPV). Typically diagnosed in older men around 70 years old, early detection remains a challenge due to the limitations and the late presentations in diagnostic tools. Late diagnosis often impacts prognosis, highlighting the need for effective biomarkers. While visual inspection aids diagnosis, tumorous may be concealed within phimosis or go unnoticed clinically, compounded by imaging limitations and the absence of specific markers. Penile carcinoma, in particular, warrants attention according to recognized histopathological and serum biomarkers. This study postulates differential marker expression in penile carcinoma based on differentiation degree and explores uncharted molecule distinctions between tumor subtypes, aiding comprehension of their biological behaviors. Moreover, proposed diagnostic biomarkers promise tailored subtype-specific approaches. The immunohistochemical analysis will profile protein expression in 34 penile carcinoma samples, encompassing subtype V, and groups P, M, and W. By the regulation of the protein expression patterns, this study aims to unravel molecular nuances underlying penile cancer subtypes, potentially revolutionizing diagnostic paradigms, and therapeutic strategies.

Keywords: Penile cancer, biomarkers, cell proliferation, inflammation, autophagy, cell cycle, IRS-4, HAT-1

P08 - Gestational venous disease drives to increased expression of extracellular vesicles markers and the NLRP3 inflammasome machinery

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Chronic venous disease (CVD) involves functional and structural changes in the venous system due to ambulatory venous hypertension, predominantly affecting the lower limns with typical symptoms and signs including varicose veins, telangiectasia and edema. Approximately one in three women develop CVD during pregnancy. Evidence suggests that CVD during pregnancy significantly stresses maternofetal structures, particularly the placenta and umbilical cord. However, the extent and pathobiological implications of these changes require further characterization. Among the mechanisms described, it has been suggested that CVD during pregnancy triggers an exacerbated inflammatory response and multiple behavioral changes in this tissue to be explored. The placenta is a major role of extracellular vesicles (EVs) during pregnancy, and previous works have demonstrated that their production are notably increased under pathological conditions. In the present work, we discuss the potential implication of 5 components associated with extracellular vesicle production: Tetraspanins -CD9, CD63, CD81-, ALG-2- interacting protein X (Alix) and the chaperone heat-shock protein (HSP-70) together with the possible alteration of the molecules associated with the NLRP3 inflammasome, an unexplored component in this entity. Having this goal, we analyzed gene and protein expression of these proteins in the placental tissue of women with CVD (n=62) and without this condition (n=52) through RT-qPCR and immunohistochemistry (IHC), respectively. Our results show a marked increase in gene and protein expression of EV-associated molecules tetraspanins (CD9, CD63 and CD81), ALIX and HSP-70 and inflammasome markers (NLRP3, ASC, caspase 1, caspase 5, caspase 8 and IL-1β) in the placenta of women with CVD. Our work gains further insights into the pathophysiological signatures of this condition in pregnancy, as well as the potential relevance of these markers in the placenta behavior and function. Future works should be conducted to deepen on the inflammatory and EV characterization of pregnant women with CVD.

Keywords: Chronic venous disease (CVD); Histopathology; Extracellular vesicles; Tetraspanins; ALIX; HSP- 70; NLRP3 inflammasome.

Histology and Histopathology
From Cell Biology to Tissue Engineering

P09 – The importance of oxytocin in psychotic disorders and the implication in placental alterations

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Psychosis in pregnancy, though rare, poses risks for both mother and newborn. First-episode psychosis (FEP) can lead to adverse outcomes, and prior studies have shown histopathological changes in the placenta of women with FEP, indicating potential impacts on maternal-fetal well- being. However, the precise role of hormones like oxytocin (OXT) and arginine vasopressin (AVP), and their receptors (OXTR and AVPR1A) in the placenta of FEP women remains unclear. Understanding these roles is crucial given the placenta's vital functions and its involvement in maternofetal programming. Psychosis encompasses various symptoms, including delusions and hallucinations, and affects multiple psychiatric conditions. Despite its complexity, the etiology of psychotic disorders involves genetic and environmental factors. FEP during pregnancy, though uncommon, is associated with significant risks and obstetric complications. Limited knowledge about its pathophysiology makes management challenging for clinicians, who must weigh the benefits and risks of treatment options. The placenta, crucial for fetal development, undergoes structural changes in women with FEP, potentially affecting fetal neurodevelopment. Hormones like OXT and AVP play essential roles in gestational processes and are expressed in the placenta. Disruptions in these systems have been observed in women with FEP, but their role in the placenta remains unclear. The current study aims to elucidate the gene and protein expression of OXT, OXTR, AVP, and AVPR1A in the placenta of FEP women compared to nonpathological controls. Using RT-qPCR and immunohistochemistry, researchers seek to understand the implications of altered hormone levels in the placenta of women with FEP, shedding light on potential mechanisms underlying maternal-fetal distress and guiding future clinical management strategies.

Keywords: Placenta, Psychotic disorders, hormones like oxytocin and arginine vasopressin.

Histology and Histopathology
From Cell Biology to Tissue Engineering

P10 - The presence of novel collagen subunits in the fibrotic scar formation after myocardial infarction: study in clinical and preclinical models

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Introduction: Myocardial infarction (MI) consists of the acute thrombotic occlusion of a coronary artery, leading to an abrupt reduction of oxygen and nutrient supply to the downstream myocardium. Following MI, a well-orchestrated inflammatory response is rapidly initiated to eliminate necrotic cardiomyocytes from the infarcted region. Afterwards, a myofibroblast-derived solid fibrotic scar formation is crucial to prevent the occurrence of more extensive left ventricular dilation and adverse cardiovascular events. Although experimental and clinical studies indicate that type I and III collagens are the main components of the post-MI fibrotic scar, RNA-sequencing data in mice submitted to permanent coronary occlusion pointed out the participation of up to 26 collagen subunits. Our objective was to pinpoint the participation of previously unreported collagens in post-infarction cardiac fibrosis in different scenarios.
Methods: Gene and protein expression of fibrillar (types II and XI) and non-fibrillar (types VIII and XII) collagen were determined in: RNA-sequencing data from 92 mice undergoing myocardial ischemia; mice submitted to permanent (non-reperfused MI, n=8) or transient (reperfused MI, n=8) coronary occlusion using qRT-PCR; eight autopsies from chronic MI patients via immunohistochemistry staining followed by morphometric analysis.
Results: RNA-sequencing analysis showed increased mRNA expression of collagen types II, VIII, and XII one day after ischemic insult, whereas type XI displayed augmented transcriptomic levels at day 3, a tendency that persisted 21 days afterwards. In reperfused and non-reperfused MI models, their gene expression was heightened 21 days post-MI induction and positively correlated with infarct size. In chronic MI patients, immunohistochemistry analysis demonstrated their presence in the fibrotic scar. Functional analysis indicated that these subunits probably confer tensile strength and ensure the cohesion of interstitial components.
Discussion & conclusions: Our data reveal that novel collagens are present in the infarcted myocardium. These data could lay the groundwork for unraveling post-MI fibrotic scar composition, which could ultimately influence patient survivorship.

P11 - Thyroid ciliopathy as an extra-renal manifestation of ADPKD

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Introduction: Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited renal disorder that results in progressive renal cyst formation, renal failure and, finally, loss of renal function [1]. ADPKD is considered a ciliopathy due to the association with abnormal primary cilia (PC) function. PC are present in the human thyroid gland extending from the apex of follicular epithelium into the lumen, where they contribute to the complex mechanism of thyroid hormone biosynthesis whose alterations haven proven to be related to functional thyroid disease [2]. Although ADPKD was originally described as the first ciliopathy and a renal disease, a few extra-renal ADPKD manifestations have been described so far suggesting that ADPKD is a systemic disease affecting multiple organ systems. Among extra-renal ADPKD manifestations, there are recent evidence with ultrasound, showing thyroid abnormalities, particularly hypothyroidism with cysts, in patients with ADPKD, likely to be associated to changes in ciliogenesis. However, there is no data in the literature analyzing the relationship between ADPKD and thyroid PC. In the current study we present the first thyroid ciliogenesis analysis in a patient with ADPKD. The patient was transplanted and, during the course of its ongoing renal disease, was diagnosed and underwent surgical intervention for multinodular goiter.
Methods: Paraffin sections of thyroid and kidney samples from the ADPKD patient were stained with H-E, and immunofluorescence for Thyroglobulin, E-Cadherin, Beta-Catenin and acetylated α- tubulin or Arl13B for ciliary axoneme. Normal thyroid and renal tissue were used as controls.
Results: The thyroid tissue of the patient showed a very heterogeneous pattern with large hypoactive follicles or “cysts” coexisting with small areas with normal-size active follicles. In both types of follicles, a marked decrease in frequency and length of PC was observed. The differences were higher in large-hypoactive follicles and appeared to be associated to a decrease in E- Cadherin and B-Catenin expression. Renal PC of the patient were generally longer than in normal kidney, but the frequency was similar than in control.
Discussion & conclusions: To our knowledge, this is the first study analyzing thyroid ciliogenesis in an ADPKD patient. Our findings show a dramatic decrease in frequency and length of PC associated to thyroid follicle structure. Loss of cilia was observed to be associated with the maintenance of the cell-cell adhesion features of thyrocytes. Our results show that thyroid ciliogenesis is affected in our ADPKD patient and, as well as in the development of other thyroid functional disorders, PC appear to have a role. Although further studies are necessary, our findings are in accordance with data in the literature, obtained by gammagraphy or ultrasound, considering thyroid hypothyroidism and thyroid cysts as extra-renal manifestations of ADPKD. If confirmed, our results suggest that ADPKD patients should be monitored and followed-up by a multidisciplinary team including thyroid endocrinologists.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P12 - New histological criteria in colorectal polyps. Clinicopathological implications

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Introduction: Colorectal cancer is the second most common tumor and the second cause of death from cancer in our country. The analysis of histopathological criteria in colorectal polyps determines the risk of lymph node metastasis and serves as a guide for the new therapeutic strategy, based on intestinal resection for high-risk patients. The objective of the study was to determine how many patients, classified as high-risk polyps, had lymph nodes affected after surgery, establishing the number of them who benefited from intestinal resection.
Methods: 73 patients, diagnosed with adenocarcinoma on a colorectal polyp, with submucosal invasion, were retrospectively studied between 2021 and 2023. High risk was defined if they met one or more of the following criteria: a) poor histological differentiation; b) submucosal invasion >1000 μm; c) Haggitt Level 4; d) deep resection margin <1mm; e) intermediate or high tumor budding; and f) presence of lymphatic, vascular and perineural invasion.
Results: In our study, 67% were classified as high risk, compared to 33% classified as medium- low risk. The percentage of lymph node metastasis after intestinal resection in high-risk patients was 11.9%. The variables that showed a more powerful relationship in stratification as high risk were the degree of tumor differentiation and submucosal invasion.
Discussion & conclusions: Patients with submucosal colorectal adenocarcinoma and high-risk criteria, who underwent intestinal resection, had lymph node metastasis in 11.9% of cases. The histopathological criteria for high-risk classification were: a) poor histological differentiation (G3);
b) submucosal invasion >1000 μm; c) Haggitt level 4; d) deep resection margin <1mm; e) intermediate (5-9) or high (>10) tumor budding; and f) presence of lymphatic, vascular and perineural invasion. Two parameters showed the strongest relationship in the stratification as high risk in the selected sample: the degree of differentiation and submucosal invasion. According to the above-mentioned variables, 67% of the patients in our study were classified as high risk, with 33% being medium-low risk. In the high-risk group, 73.5% underwent bowel resection and 26.5% underwent endoscopic follow-up. Our results are consistent with the objective that an early intervention program, based on the study of histological criteria and bowel resection in high-risk patients, may improve the rate of local recurrence and lymph node metastasis of CRC. This would result in earlier diagnosis, more accurate treatment, and a better prognosis for patients.

P13 - Development of novel oral anticancer vaccine targeting an angiogenic peptide

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Introduction: Targeting tumor-associated angiogenesis is an atractive strategy since it would produce a potent anti-tumor effect with low probability of resistance development. Angiogenesis is one of the crucial steps in tumor development and its limitation leads to reduction of tumor growth as a result of limited blood supply [1]. Here we present preliminary results on a new DNA vaccine, targeting an angiogenic peptide. The vaccine has been designed for oral delivery using a *Salmonella typhimurium* bacterial ghost carrying the DNA construct in order to break T-cell tolerance and induce long-lived T-cell anti-metastatic immunity. The angiogenic peptide of interest is Proadrenomedullin N-terminal 20 peptide (PAMP), an adrenomedullin (AM) gene-related peptide, which exhibits a potent proangiogenic potential, even at femtomolar concentrations [2].

Methods: DNA encoding murine PAMP along with two tetanus toxin epitopes (P2 & P30) as hapten [3] was inserted into the pcDNA3.1 vector and *S. typhimurium* bacteria were electroporated to achieve transformation. Bacterial ghosts were generated by treating 2.0 mL of bacterial culture with 7%(v/v) Tween80 for 24 h at 37°C, followed by adjusting pH to 3.6 with lactic acid. C57BL/6J mice were immunized by oral gavage containing bacterial ghosts transformed with either PcDNA3.1- $PAMP^{+/+}$ or PcDNA3.1-$PAMP^{-/-}$ (control) plasmids. The immunization was repeated for 3 times with 3 weeks intervals. Mice were then challenged with B16F10 melanoma cells through their tail vein. Three weeks later mice were sacrificed and lung metastases were evaluated and paraffin processed for further analysis.

Results: Success of the immunization campaign was confirmed by positive ELISA titers against PAMP20 in vaccine-treated mice whereas they were negative in the controls. Treated mice showed a lower lung weight and tumor nodule number when compared to control mice. Histology of the immunized lungs showed a significantly higher tumor infiltration of iba+ macrophages and CD3+ T-cells (Fig. 1) and reduction of α-SMA+ neo- angiogenic blood vessels in comparison with controls.

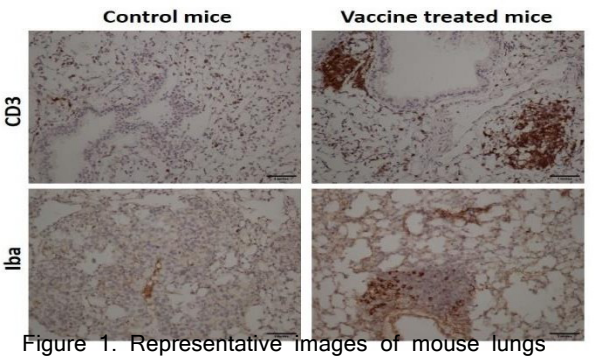

Figure 1. Representative images of mouse lungs stained for CD3 and Iba antibodies. Magnification: 200x.

Discussion & conclusions: Our proposed oral DNA vaccine encoding an angiogenic peptide has been identified as effective in reducing lung metastases through immune activation in mice, using a prophylatic model.

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P14 - Digital quantification using QuPath to improve the histological gradation of the medullary thyroid carcinoma

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Introduction: Medullary thyroid carcinoma (MTC) has different parameters to predict its clinical course. However, it was in 2022 when the International Thyroid Carcinoma Classification System (IMTCGS) was created and validated as definitive. This system classifies tumors as low or high grade based on three parameters: the presence of necrosis, a Ki67 proliferation index (Ki67PI) ≥ 5%, and a mitotic index in $2mm^2$ ≥ 5. Ki67PI is a continuous variable and due to its cut-off value, requires a precise quantification method. Visual counting (VC), commonly used in clinical practice, lacks precision and reproducibility. Digital counting (DC) resolves these issues but is still tedious and time-consuming task. Recently, AI-based platforms (PAI) emerged to enhance efficiency in quantifying the index. Due to the importance of the accurate quantification of Ki67PI, in this research, we aimed to evaluate the performance of two counting techniques, VC and PAI compared to the gold standard DC.
Methods: We studied the Ki67PI in a cohort of 26 resected MTC. The Ki67PI was quantified using three techniques: DC, VC and PAI. The Ki67PI was documented by immunohistochemistry for each case, scanned with a Philips slide scanner at 40× magnification, and quantified using the QuPath®. The same hotspots at 20x were uploaded to the IHC Expert platform for AI quantification. For each case, a minimum of 500 tumor cells were counted. Each MTC was graded using IMTCGS criteria. Results: The Ki67PI generated by both VC and AI correlates near-perfectly with the digital Ki67 index, with kappa values of 0.738 and ICC of 0.977 and 0.806 and ICC of 0.838, respectively. Encountered PAI challenges include negative or positive nuclei counted multiple times, negative or positive nuclei not counted, positive nuclei counted as negative and stromal cells counted as negative nuclei.

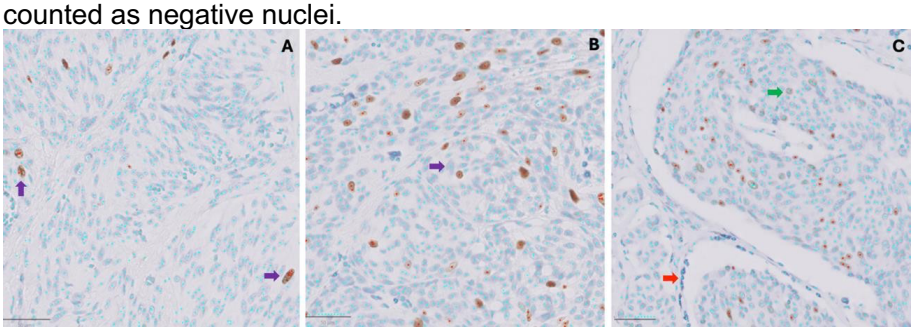

Figure 1. A,B,C. Errors of PAI quantification. (A) Positive nuclei counted multiple times. (B) Negative nuclei not counted. (C) Positive nuclei counted as negative (green arrow) and stromal cells counted as negative nuclei (red arrow).

In our MTC cohort (n=26), 30.8% and 69.2% were low- and high-grade with the IMTCGS. IMTCGS high-grade disease was significantly associated with older age (p=0.042), vascular invasion (p=0.008), and recurrence (p=0.014) as well as a decrease in both disease-free survival (HR: 19.086, p<0.001) as the global (HR: 4.107, p= 0.025).
Discussion & conclusions: The agreement between VC and PAI regarding IPKi67 digital quantification are excellent. However, the PAI makes a series of errors that underestimate the Ki67PI. High-grade MTC is associated with older age, vascular invasion, and increased risk of recurrence and has lower disease-free survival and overall survival.
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P15 - Relevance of Histology in digital pathology analysis

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Introduction: The histologist role is based on observing, recognizing and distinguishing euplastic, proplastic and retroplastic states of cells, tissues and organs [1]. Digital pathology (DP) is a technology based on the use of digitalized microscopic images of pathological tissue to extract morphometric information using software and computational tools, together with clinical-biological data often supported by artificial intelligence (AI) [2]. In DP analysis of microscopic images from oncological patient biopsies, it is essential to rigorously separate tumor from normal tissue and artefacts in order to avoid masking candidate tumor cell genetic alterations when a lot of normal tissue is present, leading to possible false negative genetic results [3].
Methods: Forty digitalized images from lung biopsies of patients from Hospital Clínico de Valencia with lung carcinoma stained with hematoxylin-eosin (H&E) were digitally analyzed with QuPath software in the framework of the national multicenter project PMP21/00107 (INGENIO). From these images, representative regions of euplastic, proplastic and retroplastic states were quantified, as well as other artefactual regions.
Results: In the studied samples it was possible to define euplastic or ortotypic state regions such as hyaline cartilage plaques, mucous glands, bronchial, bronchiolar or alveolar lining epithelium, basal membrane and pleura. Proplastic or greatest activity state was exemplified by immune cell infiltration that appeared in some subepithelial regions, the proliferation of type II pneumocytes that covered alveolar surface, fibroblast hyperplasia, anthracosis and basal membrane thickening. In some samples, retroplastic or degeneration state was present, which was associated with fibrosis. The number of tumor cells ranged from 1,700 to 116,000 (2-81% malignant epithelial cells and 19- 87% stromal tumor cells). The number of cells in eu-pro-retoplastic tissues ranged from 0 to 84,000. The regions classified as artefacts corresponded to necrosis, pinching, unfocused areas, hemorrhage and areas with crushed cell nuclei.
Discussion and Conclusions: Detailed assessment of all fragments from oncological patient biopsies is required to identify and stablish the limits of normal, tumoral and artefact regions that are often mixed, hindering the optimal evaluation of tumor representation. Furthermore, in this project, precise segmentation of tumor regions will allow to better define malignant tumor cells and its related stroma cells, which has implications for PD-L1-related immunotherapy. Therefore, DP analysis supported by the assessment of expert histologists is crucial for identifying and separating normal from tumor tissues (with the corresponding separation of neoplastic and stromal cells) and artefacts, which complements and benefits the application of AI to histopathological diagnosis, as well as therapy and prognosis of the patient. [1].

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P16 - Vitronectin secretion by 3D Ewing sarcoma and neuroblastoma model tumors in their culture media

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Introduction: Vitronectin (VN), a multifunctional glycoprotein present in plasma and extracellular matrix (ECM), plays crucial roles in cell adhesion, migration, and tissue remodeling. VN has been implicated in migration, tumor progression, and metastasis in various types of cancer. Tumor cell secretion of VN to culture media was poorly studied. Ewing sarcoma (ES) and neuroblastoma (NB) are two of the most common solid tumors in children and young adults. Due to their aggressiveness, they suppose a significant clinical challenge. Three-dimensional (3D) tumor models have become valuable tools for the development of new diagnostic and therapeutic strategies [1]. In NB, presence of VN in biopsy samples has been associated with poor prognosis [2]; however, in ES was not described. There are no plasma VN determination studies in any of the tumors. Our aim is to know the relationship between tumor size, clinical-molecular characteristics, and VN secretion to ECM and plasma in pediatric tumors.

Methods: As a first step to reach our objective, 3D hydrogels (HGs) of gelatin with tyramine and silk fibroin (25:75) were fabricated without and with exogenous added VN (0.4 mg/mL). Each HG, contained $1.25x10^5$ of commercial cells or $2.5x10^5$ of PDX (patient derived xerograph) cells, previously cultured in 2D, included in the solution. ES cell lines A673 (ATCC, USA) and PDX73, and the NB cell lines SK-N-BE(2), SH-SY5Y (ATCC, USA), PDX1, and PDX2 were cultured in HGs for 2 and 3 weeks. Culture media from monolayer cultures and HGs were collected and concentrated with SpeedVac™. VN concentration was quantified using enzyme-linked immunosorbent assay (ELISA, Bio-Techne, USA). HGs were formalin-fixed and paraffin embedded and slices were stained with Hematoxylin/Eosin. Cell density and morphometric evaluation were performed using digital analysis with QuPath.

Results: The six cell lines grew in both types of HGs scaffoldings and times, range=$4x10^5$ to $13.2x10^6$ cells. In NB-HGs, an increase in cellularity was detected at the 3rd week with respect to 2nd week of culture (mean=$4.7x10^6$ and $2.1x10^6$ respectively); opposite trend was identified in ES- HGs (mean=$6.5x10^6$ and $9.4x10^6$ respectively). The release of VN, in control HGs (no cells) with exogenously added VN at both culture times, was null. VN was detected in their culture media of all cell lines grown in monolayer and in HGs without and with added VN, except for PDX2. VN secretion per million cells was higher in HGs media than in monolayer cultures (mean = 15.8 ng/mL vs. 1.2 ng/mL).. Secretion was higher after 3 weeks of culture than after 2 weeks (mean=19.8 ng/mL vs. 10.9 ng/mL) by all cell lines except for SK-N-BE(2). Of interest, VN secretion by NB-HG was higher without VN addition than with VN (mean = 14.1 ng/mL vs. 11.1 ng/mL); in HG-ES scaffolds without VN appear to decrease secretion (mean = 18.7 ng/mL vs. 20.4 ng/mL). Overall, ES-HG were observed to produce 40% more VN than NB-HG.

Discussion and Conclusions: The secretion of VN in 2D and 3D culture media reflects a different relationship between cell growth and survival in both tumor types, being dependent on the scaffold and culture time. Studies are needed to determine the potential of VN as a prognostic biomarker in liquid biopsy.

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P17 - Women with papillary thyroid carcinoma show an increased expression of insulin receptor substrate-4 (IRS-4) with a decrease of KLOTHO after total thyroidectomy

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Papillary thyroid carcinoma is the most common and prevalent type in our environment, with a clear and marked surgical indication. It is a type of tumor that spreads rapidly. Therefore, the need arises to develop new biomarkers that allow us to monitor the effectiveness of surgical interventions, as well as serve as a prognostic value of the disease. In this sense, a retrospective cohort study is carried out in men and women patients undergoing total thyroidectomy. In the tissue samples, a protein expression study was carried out by immunohistochemistry of the proteins related to the insulin receptor substrate-4 (IRS-4) expression pathways and cell survival. Our results show how there is an increase in the expression of IRS-4, correlated with the significant decrease in KLOTHO in the tissue of women undergoing total thyroidectomy, compared to men. Our results allow us to establish an approximation, for the first time, of the difference between papillary thyroid carcinoma in men and women undergoing total thyroidectomy. All of this allows us to be the beginning of possible markers involved in the prognosis and personalized therapy of a prevalent type of tumor with clear surgical indications.

Keywords: papillary thyroid carcinoma, IRS-4, KLOTHO, woman, total thyroidectomy.

Histology and Histopathology
From Cell Biology to Tissue Engineering

P18 - Maqui berries reduce inflammation and improve damage in a chronic Crohn disease mouse model

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Introduction: Inflammatory bowel disease (IBD) is a term primarily used to refer to diseases characterized by chronic inflammation of the digestive tract, along with symptoms such as patient weight loss, diarrhea, presence of blood or mucus in stools, or abdominal pain. The literature also defines IBD as a term that mainly encompasses two diseases: ulcerative colitis (UC) and Crohn's disease (CD). In this study, a chronic CD model was used to analyze the effects of different nutraceuticals. One of these nutraceuticals is the extract of *Aristotelia chilensis* (maqui), a Chilean berry that possesses one of the highest antioxidant capacity compared to other berries.
Methods: Different therapeutic concentrations and combinations of nutraceuticals were tested on two different cell lines (HT-29 and RAW 264.7) to evaluate their effect on cell viability and ROS reduction; and maqui extract was selected for *in vivo* experiments. *In vivo* studies were performed using a chronic CD model. Groups of 6 mice were exposed to increasing concentrations of TNBS for 3 weeks, with a prior week of cutaneous sensitization. In the end, clinical scores were determined, animals were sacrificed, their organs were extracted, and several measures were taken. These organs were processed for subsequent sectioning and staining, except for the colon, which was divided into two parts, one for histological processing (histologic score) and the other for protein extraction.
Results: The *in vitro* experiments showed that there was no combination that improved the results of nutraceuticals alone. The extract of maqui in two different concentrations was finally selected as treatment for the TNBS model. Significant differences were observed among sick and healthy animals, thus validating the model. Additionally, there was a similarity in the results between healthy animals and animals treated with the higher doses of the maqui extract. Specifically, the proteins from inflammasome NLRP3 and NLRP6, analyzed in colon slices by immunohistochemistry, showed an increase in animals affected by the disease and a decrease in healthy and treated animals.
Discussion & conclusions: The results obtained demonstrated that the chronic TNBS model has been successful, due to the differences observed between healthy and sick animals. Furthermore, it has been shown that maqui extract possesses therapeutic effects in this chronic CD model, relieving inflammation and improving clinical score.

Histology and Histopathology
From Cell Biology to Tissue Engineering

P19 - Skin neoplasms in young patients (under 35 years old): study of histopathological patterns and associated risk factors

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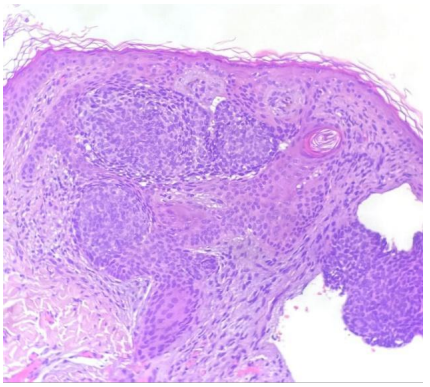
Introduction: Skin cancer represents the most frequent cause of malignant neoplasia worldwide, specifically in the Caucasian population, with the most common types being basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma, respectively. Both BCC and SCC have a good prognosis when diagnosed and treated correctly; however, in some cases, they can locally advance and metastasize. Conversely, the majority of skin cancer deaths are caused by melanoma, which represents only <5% of the total skin cancers.
Objective: To describe histological types, risk factors and clinical and histopathological data related to skin cancer in a young population.
Methods: A retrospective study was carried out that included 85 patients under 35 years of age with malignant skin lesions in the last 4 years at the Virgen de la Victoria University Hospital in Malaga. The data were obtained through a search in the database of the Pathological Anatomy Service (Vitropath) following specific inclusion criteria. Clinical and histopathological data of the lesions were collected.



Results: The most frequent lesions were basal cell carcinoma (BCC) (62.5%) and melanoma (29.5%). They affected women in a greater proportion than men (59.1 vs 40.9%). BCCs and squamous cell carcinomas (SCC) occurred most frequently in the head and neck (63.7 and 75%), with the back occupying first place in melanomas (36.4%). Surgical excision was sufficient treatment for the majority of lesions (90.9%). There were 6 distant metastases that were entirely attributable to melanoma, with the Breslow thickness being greater in those who did it compared to those who did not (p=0.001). Superficial spreading melanoma (MES) was the most frequent (69.2%), as well as T1 melanomas (34.6%) and stage IA (33.7%). There were 3 deaths due to melanoma.
Conclusions: It remains vitally important to recommend sun protection given the evidence of the emergence of malignant neoplasms in young people. Although most lesions have a benign course (BCC and SCC), melanomas have an invasive and metastatic potential that can complicate the patient's prognosis.

Histology and Histopathology
From Cell Biology to Tissue Engineering

P20 - Immunohistochemical expression of CCR9 in normal human pancreas and pancreatic adenocarcinomas

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Introduction: The global incidence of cancer is increasing due not only to the greater longevity, but also to the alarming increase among young adults. Pancreatic cancer (PC) is the malignant disease with the highest mortality rate, with 511,992 new cases reported in 2022, causing over than 467,000 deaths [1]. Among the reasons for this lethality, late diagnosis and resistance to chemotherapy play a key role. Immunotherapy represented a revolution in the treatment of many types of cancer, with a significant improvement in patient's prognosis. However, current immunotherapeutic drugs in PC have not shown clinical benefits, highlighting the need to characterize novel therapeutic targets for development of new therapeutic agents. CCR9 (Chemotactic Cytokine Receptor 9) is physiologically expressed on immature T lymphocytes and on a small population of circulating lymphocytes [2]. The expression of CCR9 or its only ligand (CCL25) has been associated with an increased metastatic capacity and tumor chemoresistance. Specifically in PC, the expression of CCR9 enhances cell proliferation and exerts strong immune-modulatory effects on T cell responses. The aims of this study were to determine the expression of CCR9 in normal pancreas and PC and to correlate findings with clinicopathological characteristics of tumors.

Methods: A total of 60 pancreatic adenocarcinoma samples, retrieved from the Pathology Department files of the University Hospital (Santiago de Compostela), were analyzed. After testing 7 different commercial primary antibodies, paraffin sections were automatically immunostained in an AutostainerLink 48 (Agilent), using rabbit monoclonal 4G2 anti-CCR9 antibody (Abcam AB288375, Cambridge, UK), EnVision®+ Dual Link System-HRP as detection system, and DAB as chromogen. The immunohistochemical results were evaluated as: 0 (no immunostaining), 1+ (weak), 2+ (moderate) and 3+ (high).

Results: In normal exocrine pancreas we observed weak to moderate plasma membrane expression for CCR9, both in acini and ducts (Fig. 1A). CCR9 immunoreactivity was found in 43/60 pancreatic adenocarcinomas studied (72%). Plasma membrane immunostaining was absent (0) in 17 cases (28%), weak (1+) in 25 (42%), moderate in 11 (18%), and high in 7 (12%) (Fig.1B).

Discussion and Conclusions: The current study demonstrated a high expression of CCR9 in PC and its correlation with greater probability of distal recurrence. Our results could contribute to development of a novel immunotherapeutic strategy for PC based on first-in-class antibodies against CCR9.

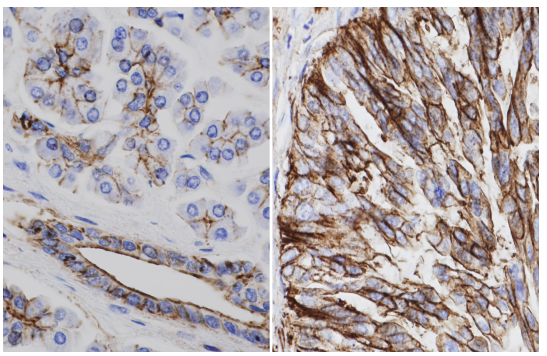

Figure 1. A) Plasma membrane expression of CCR9 in acini and pancreatic ducts. B) Intense positivity (3+) for CCR9 in a case of pancreatic adenocarcinoma (x40).

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P21 - Gastrointestinal tract changes in the offspring of the maternal immune activation model: modulatory role of N-acetylcysteine

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Background and aims. Schizophrenia (SCZ) is a complex mental disorder. Genetic and environmental studies suggest that alterations in the immune system substantially increase the risk of SCZ. The gastrointestinal (GI) tract constitutes the largest immune organ in the body and GI comorbidities occur in SCZ. The use of the mimetic viral antigen Poly I:C (Poly), as in the maternal immune activation (MIA) model, induces an SCZ-like phenotype in the offspring. Our objective was to describe the possible changes developed in the GI tract of this offspring and check whether gestational administration of N-acetylcysteine (NAC), a potent antioxidant, could prevent GI alterations in this MIA model.

Methods. On gestational day 15, pregnant Wistar rats received an intravenous (iv) injection of Poly (4 mg/kg) or Saline; half of the dams received NAC (500mg/kg) in the drinking water for 7 (NAC7) or 21 days (NAC21). Six groups of adult male offspring were used: Saline, no NAC (Control, n=9); Saline, NAC7 (CN7, n=7); Saline, NAC21 (CN21, n=8); Poly, no NAC (Poly, n=9); Poly, NAC7 (PN7, n=10); Poly, NAC21 (PN21, n=8). At postnatal day 90, animals underwent evaluation for general GI motility using radiographic methods for 24 h. GI transit was evaluated semi-quantitatively, and size and content density were determined for each GI organ along the radiographic session. After sacrifice, the GI tract was obtained and photographed for subsequent macroscopic analysis using ImageJ software. Then, samples of the distal colon (DC) were collected for immunohistochemical evaluation of immunocytes (anti-CD163 for M2 macrophages, anti-MPO for neutrophils, anti-CD3 for lymphocytes).

Results. In the X ray study, Poly-offspring showed slightly accelerated intestinal transit, that tended to normalize when NAC was administered throughout gestation. In addition, Poly- offspring had the smallest and densest cecum and fecal pellets, whereas NAC treatment, especially for 7 days, increased area and density in both control and Poly-offspring. In the stomach, the only observed finding was a slight delay in emptying and a slightly bigger size in NAC21-treated groups. The enlargement of the stomach in NAC21-treated groups was confirmed in the macroscopic study and was significant for Poly. With respect to immune cells, neutrophils were more abundant in Poly and CN7 but this was slightly reduced in PN7, with no significant differences. As for M2 macrophages, NAC reduced their presence in the DC, irrespective of the treatment, being significant in the PN21 group. The presence of CD3 lymphocytes in the three Poly-groups was significantly reduced compared to Control. However, NAC increased the amount of CD3 lymphocytes.

Conclusions. The findings of this study confirm the presence of both functional and immune GI alterations in the MIA model, which may be modified by short- and long-term NAC- treatment during gestation.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P22 - *Ex vivo* and *in vivo* characterization of new nerve artificial conduits generated by 3D printing and natural biomaterials

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Introduction: Peripheral nerve injuries have a high morbidity and prevalence in our society, thus new alternatives to solve these problems are needed [1]. In this sense, 3D printing is a promising tool to generate scaffolds and 3D structures for tissue engineering applications. Therefore, in this work we generated, by printing technology hollow 3D PLLA conduits and, subsequently, they werefunctionalized with different fibrin-based hydrogels.
Methods: Mesh-patterned PLLA conduits (20x3 mm) were generated by solvent-cast direct writing3D printing [2] and functionalized with fibrin (F-PLLA), fibrin-agarose (FA-PLLA) and 0.1% genipin-crosslinked FA (FAGP-PLLA) hydrogels. Compression properties were tested, and biological properties were evaluated by metabolic and cell viability test by using human neural derived cells (SH-SY5Y). Finally, after subcutaneous implantation in Wistar rats, *in vivo* biocompatibility of eachproduct was assessed after 10 weeks. Samples were harvested and histologically analysed including immunohistochemistry for pro-regenerative macrophages (CD206+).
Results: The biomechanical results showed few differences in the load values in the compressiontest, with higher viscoelasticity results in the functionalized experimental groups. Concerning the metabolic and viability results, the application of fibrin-derived hydrogels showed an improvement in the values of these tests compared to PLLA alone, being especially superior in the FAGP-PLLAgroup. Finally, *in vivo* histological biocompatibility analyses confirmed a greater preservation of the circular geometry of FA and FAGP functionalized hybrid conduits. In addition, a high presence of pro-regenerative macrophages was observed in all experimental groups, as well as the formation of new connective tissue around and inside the implanted scaffolds.
Discussion & conclusions: The application of functionalization protocols based on fibrin hydrogels, especially FA and FAGP, resulted in a clear improved of the biological properties in vitroand enhanced the structural preservation *in vivo*, with optimal biocompatibility in all groups. Theseresults support the hypothesis that 3D printed scaffolds combined with natural hydrogels could bea promising alternative to generate new nerve conduits based on this technology.
Fundings: This research was financed by grant PI23/00337, funded by Instituto de Salud Carlos III (ISCIII), Ministry of Science, Innovation and Universities (Plan Estatal 2021-2023, and Plan de Recuperación, Transformación y Resiliencia). Co-funded by the European Union, Fondo Europeode Desarrollo Regional ERDF-FEDER. Funded by grant CPP2021-009070 by “Proyectos de colaboración público-privada”, Plan de Investigación Científica, Técnica y de innovación 2021- 2023, Ministerio de Ciencia e Innovación, Unión Europea, Agencia Estatal de Investigación, España.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P23 - Neuronal degeneration and glial activation precede vascular impairment in the retinas of human donors with diabetes

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Introduction: The predominant cause of vision impairment among individuals with diabetic retinopathy (DR) has traditionally been related to vascular degeneration, overlooking the potentialinvolvement of neuronal dysfunction. Although some imaging studies using optical coherence tomography have suggested structural alterations, and functional studies have shown retinal abnormalities within diabetic patients lacking retinopathy [1], the neuronal alterations in human retinal cells are unknown. This research aimed to evaluate the morphology of retinal cells in the macula of human donors with diabetes, with or without retinopathy.
Methods: A comparative cross-sectional analysis of human donor retinas from patients with diabetes or DR (n=8) and a control cohort (n=7) was conducted. Specimens proceeded from the University Hospital of San Juan (Alicante). Eyes from diabetic or DR-afflicted human donors and controls were processed to obtain transversal sections in the macula or flat-mount retinas. Neuronalcells and their synaptic connections, along with glial cells, were assessed through immunohistochemistry and confocal microscopy. The density of bipolar cells, horizontal cells, andthe thickness of the inner plexiform layer were quantified at various locations close to the fovea. The density and the area occupied by the microglia were quantified across different vascular plexuses in the central retina. Statistical analysis were performed using GraphPad Prism 9.
Results: Mean age of diabetic donors without DR (n=5), with DR (n=3) and control group (n=7) were 66±9 years, 54±13 years and 66±7 years, respectively. At the macular level, cone photoreceptors elongated their axons to maintain synapses with bipolar and horizontal cells aroundintraretinal cysts. The density of cone bipolar cells significantly decreased in diabetic and DR retinas at the foveal depression ($p<0.0001$). Impairment of synaptic connectivity between photoreceptors, bipolar and horizontal cells using Bassoon antibody was found. Rod bipolar cells exhibited alterations in the dendrites and axon terminals in diabetic and DR groups. The thicknessof the inner plexiform layer decreased in DR ($p<0.0001$). The density of the horizontal cells showeda significant decrease in DR retinas compared with control and diabetic retinas ($p<0.0001$). Thereis a sprouting of horizontal cell dendrites into both outer and inner retinal layers in DR, with a lesserextent seen in diabetic retinas. Thickening of cell body of Müller cells and increased presence of astrocytes around vessels were observed in all diabetic patients. Complete swelling of Müller cellswas only detected in advanced stages of DR. In addition, the activation of microglia was indicatedby the retraction of their ramifications and thickening of the cell, specially at the superficial vascularplexus. Microglial cell density was similar between groups; however, the area occupied by microgliain the superficial plexus was greater in the DR group compared to the diabetic group ($p<0.05$). These glial cells migrated toward specific locations of retinal blood vessels without alterations, microaneurysms and some neovascular structures.
Discussion & conclusions: Our results highlight the relevance of analyzing the synaptic connectivityin diabetic patients without retinopathy and suggest that this neuronal degeneration may contributeto vision impairment in diabetic and DR patients. Changes in the Müller cells would indicate early-stage activation and could correlate with the observed increase in the inner nuclear layer in some diabetic patients, using optical coherence tomography [2]. Moreover, the activation of microglial cells and the response of astrocytes suggests an inflammatory process in diabetic individuals. Therefore, degeneration of retinal neurons and disruption of synaptic connectivity were observed in diabetic patients preceding clinical vascular changes and neuroprotection could be an option toprevent visual impairment in DR.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P24 - Cellular and molecular dysfunction in the retina and optic nerve asan early biomarker of Parkinson's patients.

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Introduction: Patients diagnosed with Parkinson's disease (PD) often exhibit non-motor symptoms that manifest earlier than motor disturbances. Among these manifestations, there is clinicalevidence indicating visual impairments within the pathology. In particular, these non-motor symptoms include impairments in color vision, visual acuity, contrast sensitivity, and motion perception, alongside abnormalities observed in electroretinograms (ERG) and visual-evoked potentials (VEP).
Methods: Human eyes from both control and Parkinson's disease (PD) donors were used in this study. Immunohistochemistry and confocal microscopy were used to assess the density of dopaminergic amacrine cells, starburst amacrine cells, and their synaptic contacts in whole-mountretinas. The morphology, quantity, and distribution of microglia cells in optic nerve cryosections andretinal whole mounts were analyzed using bioinformatics software. Optic nerve cryosections stained with hematoxylin were morphometrically analyzed, and we quantified by immunohistochemistry the total number of axons and the number of mitochondria. Transcriptomicanalysis of the neural retina from both groups was conducted using total RNA sequencing, followedby differential expression analysis using DESeq2. Gene-disease associations were investigated using the DisGeNet platform, and enrichment analysis for affected biological processes and functional pathways was conducted using the EnrichR website.
Results: In PD retinas, we observed a significant reduction in the number of dopaminergic amacrinecells and their synaptic connections with AII amacrine cells. Additionally, within the ganglion cell layer, we've detected the accumulation of phosphorylated alpha-synuclein in axons and dendrites,alongside the presence for the first time of Lewy bodies in the retina. Also, there was noted degeneration of starburst amacrine cells, crucial for motion direction selectivity, evidenced by a decrease in their density. Notably, we identified in healthy control retinas that dopaminergic amacrine cells establish connections with choline acetyltransferase positive cells, which diminish in PD. Furthermore, microglia cells changed from their typical ramified morphology to an activatedmorphology in PD. Moreover, PD optic nerves exhibited an increase in total nerve area and in the number of bundles, along with a decrease in the number of axons and mitochondria. Finally, the transcriptomic analysis indicated that some genes are differential expressed affecting different biological processes including an impairment in the mitochondrial function and in GABA synthesis.
Discussion & conclusions: In conclusion, our findings suggest that the loss of retinal dopaminergiccells and their synaptic connections with AII and starburst amacrine cells in PD may potentially explain the visual disturbances associated with the pathology. Furthermore, we have detected molecular changes in several pathways linked to retinal cell functions, suggesting an ongoing degenerative process in these cells. Our results also indicate neuroinflammation with involvementof microglia in the retina and optic nerve of these patients. Finally, we propose utilizing the in vivo assessment of optic nerve diameter with imaging techniques, alongside neurophysiological tests evaluating motion perception, as early biomarkers of the pathology.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P25 - The histone deacetylase 6 inhibitor Tubastatin-A is neuroprotective after severe brain injury in neonatal rats

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Background: Perinatal hypoxia-ischemia (HI), a birth-related injury in neonates, occurs when blood oxygen concentration decreases, when blood flow is reduced, or when both occur simultaneously.This pathology is one of the major causes of severe disorders in newborns, and can cause death in the most severe cases. Further, more than 50% of the survivors may suffer severe neurologicalconsequences for the rest of their lives, such as motor and/or mental impairment. Using a preclinicalmodel of severe neonatal HI in rats, we wanted to explore the therapeutic effect of the histone deacetylase 6 inhibitor Tubastatin-A (Tub-A).
Methods: Fifty-one 7-day-old Sprague Dawley rats were randomly assigned to: i) HI (left common artery ligation + 150min 8%O_2/92%N_2; n=24); ii) HI + Tub-A (25mg/Kg; one i.p. dose immediately after HI and three more doses spread over the next 72 hours; n=13); iii) Sham (without HI; n=14). Seven days after the injury, the animals were anaesthetized and sacrificed for neuro-histological studies. Cortical and hippocampal brain infarction was evaluated using a well-stablished neuropathological score by hematoxylin-eosin staining followed by image analysis. Quantitative analysis of the immunohistological pattern of Myelin Basic Protein was used to determine white matter injury. Cell proliferation was evaluated by Ki67 quantification in the hippocampal neurogenicniche of the dentate gyrus. Statistical analysis was performed by GraphPad 8.0.1 and $P<0.05$ considered significant.
Results & conclusions: Animals receiving Tub-A showed significant neurohistological improvements over non-treated HI rats: hippocampal neuropathological score was reduced (3.69 vs 7.54; p=0.024), together with maintained cortical (0.83 vs 0.65; p=0.0118) and hippocampal (0.75 vs 0.42; p=0.0072) areas. Further, the amount of white matter in the cingulum was significantly higher (0.98 vs 0.63; p=0.0067) in Tub-A treated rat pups, as well as in the external capsule (0.83 vs 0.48; p=0.0214). Finally, animals that received Tub-A showed a higher number ofproliferating cells compared to non-treated HI animals (505 vs 266 cells per mm^2; p=0.0227). In a preclinical model of perinatal asphyxia in rats, these results suggest that the treatment with Tub-Amay be beneficial in cases of severe neonatal HI.

Acknowledgement: Basque Government (PIBA_2023_1_0022); Programa Investigo funded by EU-Next Generation EU (PIFINVE22/14); UPV/EHU (GIU21/054).

P26 - Histological study of the combined therapy of melatonin and therapeutic hypothermia in neonatal rats exposed to perinatal asphyxia

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Introduction: Perinatal insults like hypoxia-ischemia (HI) may produce brain injury, leading to neurological life-long disabilities or even death. The only available clinical treatment is therapeutichypothermia (TH), but it is ineffective in approximately half of cases. Melatonin has emerged as a potential therapy against HI due to its neuroprotective properties, but its role administered aloneor in combination with TH is still under evaluation. The aim of this work was to assess histologically the neuroprotective effect of combining Melatonin together with TH using a model of HI in neonatal rats.
Methods: On postnatal day 7, a total of 63 Sprague Dawley rats were subjected to ischemia (left common carotid artery ligation) followed by hypoxia (120min, 8%O_2/92%N_2). After HI, rat pups received the hypothermic treatment (32.5-33ºC for 3h) or/and Melatonin (i.p. 15mg/kg, 5min after HI, and repeated at 24h and 48h). Non-operated animals served as sham animals. The resulting groups were the following: Sham (n=10), HI (n=15), HI+TH (n=13), HI+MEL (n=15), HI+MEL+TH (n=10). At P14, the neuroprotective effect of the treatments was analyzed by measuring brain infarct through the ratios of the hemispheric and hippocampal areas and by a global and regional neuropathological score. Statistical analysis was performed by GraphPad 8.0.1 and $P<0.05$ considered significant.
Results: The HI group showed great tissue loss, thus obtaining the lowest hemispheric ratios (0.41±0.22). A slight recovery was observed in the HI+MEL (0.58±0.22) and in the HI+MEL+TH (0.63±0.19) groups, with HI+TH rats obtaining the highest hemisphere ratios (0.75±0.23; $P<0.05$ vs HI) and similar to Sham values (1.01±0.02). Hippocampal ratios for non-treated HI rats were 0.20±0.17. This hippocampal tissue loss was reduced when administering the treatments: HI+MEL (0.43±0.26), HI+MEL+TH (0.40±0.28) and HI+TH (0.51±0.40). Global and regional
(cortical and hippocampal) neuropathological scores revealed that non-treated HI animals suffered grey matter damage (global: 19.93±2.60; cortical: 8.27±1.44; hippocampal: 8.87±0.52). Aminimal recovery was observed in the HI+MEL (global: 16.73±7.70; cortical: 6.93±3.65; hippocampal: 7.20±3.32) and HI+MEL+TH (global: 17.60±6.55; cortical: 7.60±2.55; hippocampal: 7.60±2.80) groups. The HI+TH group presented the best score values (global: 12.00±9.11; cortical: 4.69±4.09; hippocampal: 5.69±3.86).
Discussion & conclusions: When compared to TH alone, we found no further histopathological benefit with the combined therapy of Melatonin with TH. This medium-term study emphasizes a need of a better understanding on how Melatonin may act together with TH for the treatment of neonatal HI.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P27 - Correlative X-ray microscopy for comprehensive analysis of ocular structures

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Introduction: Imaging techniques are essential for elucidating the intricate morphology of healthy organs and pathological changes, particularly within the visual system. Understanding healthy anddiseased states within the eye requires visualizing intricate structures across various scales. Traditional histological methods like electron microscopy and conventional sectioning encounter challenges in integrating macroscopic and microscopic observations. The integration of X-ray, fluorescence, and brightfield microscopy presents a promising approach. This study aims to investigate the efficacy of X-ray microscopy (XRM), specifically utilizing the Xradia Zeiss Versa 510, in a comprehensive examination of ocular structures such as the retina, cornea, trabecular mesh, and choroids across various physiological and pathological contexts.
Methods: Eyes were enucleated and immersed in 4% paraformaldehyde for fixation, followed by osmication with osmium tetroxide. After fixation, samples were dehydrated using a series of ascending ethanol solutions and subsequently cleared in xylene. Following dehydration, samples were embedded in paraffin. For brightfield microscopy, paraffin slices from eyes without osmium were cut and stained with hematoxylin-eosin.
For fluorescence microscopy, eyes were fixed with 4% paraformaldehyde, cryoprotected, and cut with a cryostat. Slices were incubated with primary antibodies against recoverin (rabbit polyclonal,1:500; Sigma Aldrich) and protein kinase C (mouse polyclonal, 1:300; Santa Cruz Biotechnology) overnight at 4 °C. Specific secondary antibodies were conjugated with Alexa Fluor 555 and 488 (1:100; Thermo Fisher Scientific) and counterstained with the nuclear stain Hoechst 33342 (Thermo Fisher Scientific). Brightfield images were scanned with Motic Scan (Motic DS Assistant), while fluorescence images were acquired with a Zeiss Observer Z1 microscope equipped with an Apotome module.
During the X-ray microscopy processing, the eyes were fixed with 4% paraformaldehyde and embedded in paraffin. Subsequently, they were osmicated with osmium tetroxide, dehydrated, andthen embedded in paraffin. X-ray imaging was performed using the Zeiss Xradia 510 Versa system(Carl Zeiss X-ray Microscopy, Inc.), operating at 40 kV with 1601 projections over a 360° sample rotation from a paraffin-embedded eye block.
Results: Comparative analysis between XRM images, fluorescence, and optical microscopeimages highlighted significant advantages in terms of time efficiency and cost-effectiveness. XRM facilitated high-resolution imaging, enabling detailed visualization of ocular structures such as the retina, cornea, trabecular mesh, and choroids. Its non-destructive nature preserved tissue integrityfor further analysis. This non-destructive imaging preserved tissue integrity for further analysis. Correlating XRM data with fluorescence and brightfield microscopy yielded a comprehensive understanding of ocular structures across scales. XRM visualizations accurately depicted cellular details observed in fluorescence microscopy, with specific cells labeled, showcasing XRM's capacity to capture comparable information without extensive sectioning and staining requirements.
Discussion & conclusions: X-ray microscopy emerges as a powerful tool for investigating eye pathology, surpassing traditional histology and electron microscopy methods. Its non-destructive nature and ability to image the entire eye revolutionize our comprehension of ocular diseases by enabling 3D reconstruction of the tissue architecture. The unique advantages of X-ray microscopyin visualizing and analyzing microstructures make it indispensable in histological research and diagnostic applications, offering a comprehensive approach to studying ocular pathologies and advancing our understanding of eye diseases.

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P28 - Enhancing Nerve Tissue Evaluation: Automated Morphometry with Brightfield Microscopy and Machine Learning

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Introduction: Our laboratory is dedicated to advancing the field of image analysis, particularly as itrelates to the study of sciatic nerve morphology. Our research involves a wide range of techniquesand methodologies aimed at unravelling the intricacies of nerve structure and function. One of themain tools we use is electron and brightfield microscopy, a versatile imaging modality that provides high-resolution images of biological specimens. Coupled with sophisticated machine learning algorithms, brightfield microscopy allows us to perform detailed morphometric analyses with unprecedented accuracy and efficiency. We aim to investigate various aspects of sciatic nerve morphology, with a particular focus on understanding the role of myelination. Myelination, the process by which axons become wrapped with myelin sheaths, is a fundamental aspect of nervoussystem development and function. It plays a crucial role in enhancing the speed and efficiency of neuronal signalling, thereby facilitating proper neural communication.

Methods: To study the dynamics of myelination in peripheral nerves, we employ a variety of techniques, including brightfield and electron microscopy. By analysing brightfield microscopy images of nerve tissue, which are of semi-thin sections (1 µm) stained with toluidine blue, we canquantify key morphological parameters such as axon diameter, myelin thickness, and the total number of myelinated fibres. We automate our analysis using machine learning tools such as Ilastik, enabling efficient identification and measurement of myelinated nerve fibres. Leveraging pixel and object classification capabilities in Ilastik [1], we achieve comprehensive segmentation ofnerve structures. Automated algorithms in Fiji [2] further enhance our analysis. By employing machine learning algorithms, we predict myelin homeostasis and identify molecular mechanisms underlying myelin development an remyelination. Integrating these tools streamlines our researchprocesses and enables comprehensive analysis of peripheral nerves.

Results: Our analyses have provided significant insights into the dynamics of myelination. By usingbrightfield microscopy images, we have quantified key morphological parameters and automated our analyses with machine learning tools. This approach has enabled comprehensivesegmentation and detailed morphometric analysis of nerve tissues. Our findings highlight the developmental trajectory of myelination and its regulation by intrinsic and extrinsic factors. In contrast to electron microscopy, which requires specialized equipment and expertise, brightfield microscopy allows for much shorter sample preparation and image acquisition times. However, forultrastructure that require higher magnification, we also have an electron microscope in our laboratory. This allows us to study and obtain superior quality images to which we also apply imageanalysis techniques. Some of these techniques include machine learning algorithms such as Ilastikfor classification and AimSeg [3], a Fiji plugin, for quantification and obtaining measurements suchas structure area. In particular, these algorithms are employed to quantify areas of the axon, innertongue, and myelin, thus calculating the g-ratio to study peripheral nerves.

Discussion & Conclusions: The integration of advanced imaging techniques, computational modelling, and machine learning has streamlined our research processes, enhancing our understanding of sciatic nerve morphology. Brightfield microscopy's versatility and accessibility make it a valuable tool, complemented by electron microscopy for detailed structural analysis. Our multidisciplinary efforts continue to unravel the complexities of nervous system development and function, paving the way for deeper insights into nerve regeneration and the molecular mechanismsunderlying myelin development.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P29 - Safety and Efficacy Assessment of MECA Electrodes for Peripheral Nerve Stimulation

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Recent advances in central nervous system neuroprosthetics have reignited scientific interest in neuroprosthetics and functional electrical stimulation (FES) techniques for peripheral nerves. These techniques aim to restore lost or impaired motor and sensory functions in disabled patients [1]. In order to properly develop these techniques, it is essential to carry out exhaustive studies on safety, efficacy and histological and structural consequences of stimulation and electrode implantation in peripheral nerves. Furthermore, these safety studies must be specific to the electrode type, since different electrode designs and materials may have different effects on nervetissue over time [2]. Therefore, the present work aims to study the safety limits and efficacy of stimulation for the specific case of extraneuronal and minimally invasive MECA (Multi Electrode Cuff Array) electrodes implanted in rat sciatic nerve.

For that purpose, a series of experiments were conducted to evaluate the histomorphological and ultrastructural consequences of prolonged electrical stimulation (continuous stimulation for 4 hours)and chronic electrode implantation (2 weeks) in rat sciatic nerves. Three nerve groups were compared: control, sham (implanted but not stimulated), and chronic stimulation (implanted and stimulated). Stimulation intensities were determined based on 50%, 100%, or 150% of the minimumcurrent capable of maximizing the alpha component of the spinal compound action potential (αCAP). Following the experiments, stimulated and non-stimulated nerves were histologically processed.

The results revealed the presence of mechanical damage in the nerve due to the electrode implantation, as the sham samples presented a decreased population of functional fibers, and an increased presence of damaged fibers. However, it was observed that the use of low and safe stimulation intensities, such as 158 μA, mitigated tissue damage caused by implantation. Additionally, for intensities considered safe according to literature (< 3-4 μA), although consideredunsafe according to αCAP criteria (>100% considered unsafe), those samples stimulated with 392μA (150% αCAP) exhibited fibers with a lower amount of myelin, although other morphometric parameters such as fiber area did not reflect additional signs of damage. Nevertheless, the higheststimulation intensity, 1500 μA (150% αCAP), showed the greatest presence of degenerated fibers.

These findings contribute to understand the relationship between stimulation parameters and potential long-term tissue damage. However, further studies are needed to establish safe and effective protocols, and to define a valid safety criterion regarding stimulation parameters for peripheral nerves.

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Poster Presentations

Session 2

General Histology
Histology and Reproduction

Histology and Histopathology
From Cell Biology to Tissue Engineering

P30 - Changes in the frequency and length of primary cilia according to thyroid follicle activity

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Introduction: Primary cilia (PC) are present in the human thyroid gland extending from the apex of follicular epithelium into the lumen [1], where they likely sense the colloid environment and contribute to the complex mechanism of thyroid hormone biosynthesis. In global, we have detected PC changes according to different functional thyroid pathologic states (nodular hyperplasia and Graves' disease), but we have been unable to find any direct relationship between PC and the size of thyroid follicles or the height of follicular epithelium as signs of follicular activity [1]. A possible explanation may be the intrinsic heterogeneity of the thyroid gland in relation to the morphology and functional activity of different follicles, being every follicle an independent morphofunctional entity, which follows its own biosynthetic rhythm under TSH influence. Therefore, in the present work, we have tried to identify different patterns of thyroid-follicle activity, considering their morphology and staining properties using classic and immunohistochemical techniques. Afterwards, we analyzed using double immunofluorescence (IF) if there exists any relationship between follicle activity and ciliogenesis.
Methods: Paraffin sections of human thyroid glands (5 normal, 2 nodular hyperplasia and 2 Grave's disease) were stained with H-E, PAS and immunohistochemistry for thyroid-specific markers thyroglobulin (TG) and TPO. Consecutive sections were immunostained by double IF to demonstrate PC -using acetylated α-tubulin for axoneme- together with TG, followed by Cy3 or Cy2-labelled IgG antibodies. To quantify the frequency and length of PC, a morphometrical analysis was carried out using a minimum of 1000 follicular cells of every follicular pattern selected from different samples. Statistical differences were tested by analysis of variance.
Results: According to their morphology and staining properties, four patterns of thyroid follicle were identified: active, hyperactive, hypoactive and empty follicles. The most valuable features considered were the epithelium height and the colloid density. We have found a statistically significant relationship between the activity of the thyroid follicle and the evolution of PC, being active follicles where the percentage of ciliated thyrocytes is the greatest (64,58±2,38), followed by hyperactive (61,63±1,55), hypoactive (42,30±1,95) and empty follicles (25,74±1,19). In relation to PC length , we have also observed significant differences among different follicular patterns, being active follicles where the longest PCs were displayed (2,38±0,97), followed by hypoactive (1,95±0,89), hyperactive (1,55±0,75) and empty follicles (1,19±0,6).
Discussion & conclusions: Our findings demonstrate the existence of a direct relationship between follicular activity and PC, being the colloid density for TG a main element to be considered in relation to the role developed by PC in thyroid hormonogenesis. PC are literally plunged into the colloid, where they may sense the increasing concentration of TG at the lumen under TSH influence. It is known that PC act as mechanosensors in different organs where fluid pass or is concentrated, as it occurs in renal tubules, intrahepatic bile ducts or the vestibular system. It makes sense that in a closed compartment, as thyroid follicles are, a specialized mechanism must exist to inform the overall TG-producing cells surrounding the lumen that this is refilled enough and, consequently, should slow down TG synthesis. This hypothetical new role of PC in thyroid is compatible to other possible functions developed by PC in regulating thyroid-hormone biosynthesis, mainly as chemosensor [2], or mediating TG endocytosis [3]. Summarizing, in thyroid, PC likely acts as a global biosensor of thyroid hormone byosynthesis.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P31 - Double immunofluorescence of primary cilia with different epithelial and thyroid-specific markers.

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Introduction: Primary cilia (PC) are antenna-like structures that project from the surface of most vertebrate cells and mediate cell signaling acting as mechanosensors, chemosensors or biosensors, depending on the tissue [1]. PCs are immotile and structurally composed of the basal body and the axoneme, according to a 9+0 pattern. One of the first organs in which PC were described was the thyroid gland, where their presence is outstanding [1, 2]. PC project from the apical surface of follicular epithelium to the lumen, where they likely sense the colloid environment and contribute to the complex mechanism of thyroid hormonogenesis. In the present work, we have analyzed several PC markers as well as different epithelial and thyroid-specific markers, to select the best possible combinations of antibodies for subsequent double immunofluorescence (IF) studies, taking in account their monoclonal (M) or polyclonal (P) origins.
Methods: Sections from formalin-fixed and paraffin-embedded normal human thyroid glands were stained with simple IF for different PC markers (acetylated α-tubulin/M, Arl13B/P and polyglutamylated tubulin/P, for axoneme; γ-tubulin/P or CAP-350/M, for basal body), epithelial- membrane markers (E-cadherin/P, β-catenin/M and prominin-1/P) and, finally, thyroid-specific markers (TG: thyroglobulin/P; TTF-1/M; and TPO: thyroperoxidase/M). Once selected the most appropriated specific antibodies conditions, we proceed to combine compatible pairs of them by double IF, considering their M or P origins, followed by the correspondent Cy3 or Cy2-labelled IgG antibodies. In some cases, we additionally tested some combination of antibodies for studying a specific human thyroid cell line (N-thy-ori) or the rat thyroid gland. Furthermore, we also tried to demonstrate PC using a classic immunohistochemical (IHC) method.
Results: Firstly, using IHC and acetylated α-tubulin as specific antibody, we could distinguish the axoneme of PC in same areas of thyroid sections but to a smaller extent than using IF. Therefore, we ruled out IHC and chose IF onwards, as was expected. Among axoneme markers, the best staining of PC in human thyroid sections was obtained using either acetylated α-tubulin or Arl13B antibodies, although the first also stained the cytoskeleton while Arl13B gave some nonspecific staining of the colloid. However, in rat thyroid gland, truncated PC were only marked using polyglutamylated tubulin. In relation to basal body markers, γ-tubulin antibody only worked in cell cultures, while CAP-350 stained either paraffin sections or cell cultures. In relation to antibodies for the three thyroid-specific markers (TG, TPO, TTF-1), they stained specifically their correspondent antigens in all materials, furthermore, they combined rather well with PC markers for axoneme. Finally, the basal and lateral outline of follicular cells in the epithelium was clearly immunostained by E-cadherin and β-catenin antibodies, while anti-prominin-1 mainly stained the apical membrane. Nevertheless, in some cases, prominin-1 exhibited a different punctuated pattern near the surface of follicular epithelium but at colloid level.
Discussion & conclusions: In our hands, when the objective was to analyze PC changes in thyroid sections, E-cadherin identified clearly the outline of every thyrocyte and combined perfectly with acetylated α-tubulin to mark PC. Nevertheless, our main finding was to be able to distinguish among different patterns of thyroid-follicle activity, combining TG and acetylated α-tubulin: TG stained the colloid to varying degrees, according to its density, as well as the cytoplasm of active thyrocytes; TPO and TTF-1, however, rather stained all thyrocytes independently of their biosynthetic activity.

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P32 - Exploring the relationship between gut microbiome and sexual dysfunctions

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Introduction: The gut microbiome is made up of trillions of microorganisms, including bacteria, viruses, and fungi, which are part of the gastrointestinal barrier. Its imbalance or dysbiosis has been related to cardiovascular diseases and certain digestive pathologies, such as irritable bowel syndrome or inflammatory bowel disease. Sexual dysfunctions are common pathologies related to cardiovascular disease [1]. Additionally, digestive dysbiosis can influence the development and progression of sexual dysfunctions.
Objective: Explore the relationship between the gastrointestinal microbiota and sexual dysfunctions, to learn more about its etiopathogenesis and be able to offer more therapeutic options.
Materials and methods: The bibliographic search was carried out in the PubMed database, selecting a total of 22 articles.
Results: Fifteen articles were found that related the digestive microbiota with erectile dysfunction, compared to only one article that associated it with female sexual dysfunction.
Discussion: The association of erectile dysfunction with the decrease of some bacteria in the digestive microbiome, such as *Ruminococcaceae* and *Alistipes*, has been demonstrated. On the other hand, female sexual dysfunction also seems to be related to the decrease in *Ruminococcaceae* and the increase in *Bifidobacterium* and Lactobacillus.
Conclusions: There are few articles in the literature that relate sexual dysfunction to the gastrointestinal microbiota, requiring more studies to demonstrate the relationship between these associations. Furthermore, doctors do not feel prepared to deal with sexual dysfunctions in consultation, and adequate training is necessary during the Medical Degree.

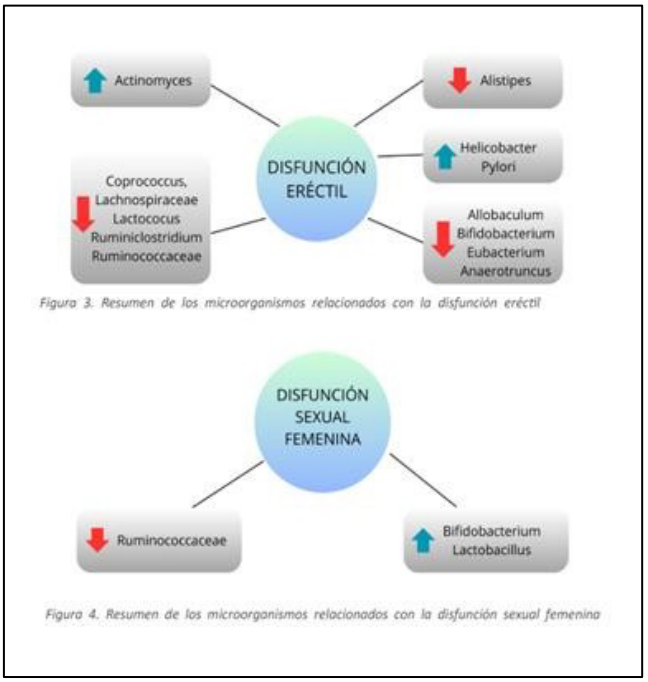

Figura 3. Resumen de los microorganismos relacionados con la disfunción eréctil

Figura 4. Resumen de los microorganismos relacionados con la disfunción sexual femenina

Keywords: gut microbiome, sexual dysfunction, erectile dysfunction, female sexual dysfunction.

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P33 - Different ultrastructural types of glycocalyx on the integument of the sessile rotifer *Limnias ceratophylli* Schrank, 1803 (Monogononta: Gnesiotrocha: Floscullariidae)

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Introduction: Rotifers are small aquatic invertebrates of high diversity (more than 2000 species) characterized by having an anterior ciliated region (corona) used for locomotion and feeding [1]. The distinctive ultrastructural feature of the phylum Rotifera is a dense intracytoplasmic lamina (ICL) in its syncytial integument. The ICL has skeletal function, and its ultrastructure has taxonomic value. The external side of the integument is a plasma membrane with a glycocalyx which varies between species [2], but, until now, no attention has been paid to the different types of glycocalyx that could exist in the diverse regions of one animal body. Considering its functional and phylogenetic interest, we have studied the glycocalyx of all integument regions of the sessile rotifer *Limnias ceratophylli* Schrank, 1803 characterized by inhabiting a pipe-shaped shell and having a rigid dorsal plate. Methods: Specimens of *L. ceratophylli* were collected in October 2022 in the Marjal of Massamagrell, Valencia, Spain. Selected samples were transferred to a slide with ~20 µl deionized water, fixed by adding a drop of 2.5% glutaraldehyde in 0.1 M cacodylate buffer at 85º C. The specimens were then transferred to a 2.5 mL Eppendorf tube and held for 45 mins. They were then washed in 0.855 mg/100 mL saccharose cacodylate, postfixed in 1% osmium tetroxide for two hours, dehydrated in a graded acetone series, and embedded in Epon 812 (Serva, Heidelberg, Germany). Serial semithin sections (1 µm) were stained with blue toluidine. Ultrathin sections (90- 120 nm) were obtained with a Reichert-Imy Ultracut ultramicrotome, contrasted with uranyl acetate and lead citrate, and examined with a JEOL 1011 Transmission Electron Microscope with GATAN ORIUS SC200 High Contrast Digital Camera. Results: In the corona, the non- syncytial regions had a hyaline glycocalyx surrounding the base of the cilia and microvilli, while the adjacent syncytial region (called the apical field) had a very thick anastomosing glycocalyx. The dorsal plate had a conspicuous glycocalyx with the appearance of brush bristles. In the rest of the trunk, the glycocalyx had a bushy appearance progressively decreasing thickness. In the foot, the glycocalyx began like that of the trunk but changed to a much thicker fibrous network at the level of the attachment peduncle (Table 1). Discussion & conclusions: Specific proteins related to glycosylation in the integument of rotifers [3], suggests that it is a relevant component. The ultrastructure of the *L. ceratophylli* glycocalyx varies in different regions of the body. It implies that comparison between species for phylogenetic purposes must consider what body region is compared. The glycocalyx of the thin ICL syncytial regions of the corona would be related with male-female recognition in mating, while the function of the other types of glycocalyx is more speculative. They would be related with the flow regime of water currents produced by the corona, with the formation of the basal region of the tube, with the defense against microorganisms, or with protection of the apical syncytial membrane when the animal extends and retracts into its tube.

Table 1. Differential traits of the external glycocalyx of *Limn ceratophylli* by body regions. Figures represent thickness in nn

Body region	Morphology	Glycocalyx	IC
No syncytial, ciliate regions	Hyaline	500	-
Apical corona field	Anastomosed	800	1(
Dorsal plate	Brush-like	300	5(
Trunk	Bush-like	200-100	400

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P35 - Effectiveness of laser photobiomodulation and Ulmo Honey treatment in the repair of wounds from deep burns in animal model: stereological analysis

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Introduction: Burns are a major public health problem that can determine physical and psychological sequelae in individuals. Thermal burns are the most common form, with flames and overheated subtances being the main agents. [1,2]. The animal model is currently used to understand the effects of new therapies, allowing macroscopic and microscopic analysis. The objective was to analyze the effectiveness of laser photobiomodulation therapy (PBMT) associated with ulmo honey (UH) in the process of repairing wounds by total thickness deep burn. We evaluated neovascularization, fibroblast proliferation, collagen fiber deposition and wound retraction rate. Methods: Este studio ha sido aprobado el el comité científico Ufro, folio nª120/123, We used 48 Sprague-Dawley rats, females between 200-240 g. We performed a burn injury in dorsal region with metal instrument, diameter: 2.0 cm, weight 30 g. The plate was heated in hot water with until boiling and placed for 30 seconds resting only the weight of the plate. After induction of the burn wound, the animals were randomly divided into 4 experimental groups: G1- Control group (without treatment), G2- PBMT-treated group, G3- UR, G4- PBMT + UR. For the treatment with PBMT we used an infrared semiconductor laser GaAlAs, 820 nm (MMOptics), spot 0.03 cm^2, 100 mW, energy density per point of 20 J/cm^2, 6 s/point, grade technique. We performed the analysis of the wound retraction rate on days 7 and 14 after the burn wound. We collected samples from the wound and adjacent tissue for histological processing with Heneghan blue staining. For the stereological analysis, the epidermis was chosen as a reference plane and cuts perpendicular to this foreground were made and the uniform vertical randomization (VUR) was generated. We analyze blood vessel surface density collagen fiber volume density, and fibroblast number density. We used the ANOVA test, with Tuckey's post-test considering a significance threshold of 5%, in R Studio software, version 2023.09.0+463.

Results: The burn wound area decreased on days 4 and 7 after injury as shown in **Fig. 1**. PBMT and PBMT + UH showed a greater decrease in the area of the burn injury, but the result was not significant (p=0.269). We are anticipating a greater healing of the burn wound among the group treated with PBMT + UH, with an increased of angiogenesis, fibroblasts, and a better deposition of collagen fibers. Discussion & conclusion: Previous studies have shown that PBMT and the UH treatment are effective in increasing angiogenesis, deposition of collagen fibers and increasing the wound retraction rate. However, there is no evidence to support the benefits of these therapies when combined.

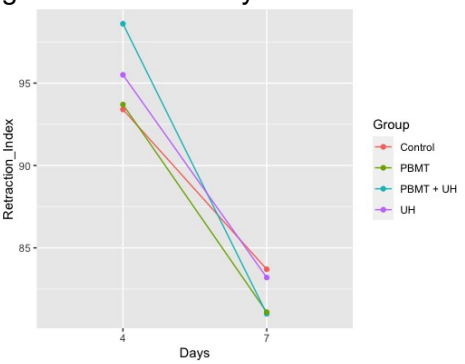

Fig. 1. Wond retraction rate (%) for 4 and 7 days.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P36 - Developmental appearance of oligodendrocytes in the developing chicken visual system

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Introduction: Myelination of retinal ganglion cell axons in birds takes place during the embryonic period [1]. The oligodendrocyte precursors migrate from the medial ventricular layer of the ventral wall of the third ventricle along the optic chiasm. At the same time, they progressively become differentiated cells, and enter the retina through the optic nerve [2, 3]. The present study aims to analyze the timing of myelination of the optic axons during avian visual system development by using two immunohistochemical myelination markers: myelin basic protein (MBP) and proteolipid protein (PLP).
Methods: Chicken embryos from E12, E14, E16, E18 and P0 were used. They were fixed in PFA, included in gelatin, and subsequently frozen at -80 ºC. The sections obtained were 20 µm. Immunohistochemical studies were carried out to detect MBP and PLP, and the images were obtained in the fluorescence microscope with an attached Leica camera. The images were treated with Photoshop CS4 for the assembly of the figures.
Results: MBP was first detected in the optic chiasm at E12 whereas the onset of PLP labeling starts at E14. From this stage, the immunochemical signal for both myelin proteins spreads from proximal to distal regions of the optic nerve. The first MBP-positive oligodendrocytes are detected in the optic nerve head at E14, while PLP immunosignal is delayed until E16. From these stages onward, MBP- and PLP-immunoreactivity are restricted to the optic fiber layer of the retina and extend from central to peripheral regions. In this sense, the first MBP-positive oligodendrocytes are detected at E16 in the peripheral retina.
Discussion & conclusions: These results show that MBP is immunohistochemically detected earlier than PLP. Furthermore, there is a proximal-to-ventral wave of oligodendrocyte differentiation in the optic nerves and a central-to-peripheral dispersal of immunoreactive oligodendrocytes in the developing avian retina.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P37 - Identification of PBX1 as a new putative biomarker for Limbal Epithelial Progenitor Cells in human cornea

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Introduction: The human cornea is a highly dynamic tissue with huge regenerative capability to ensure proper corneal function and maintain its structure. Limbal Epithelial Progenitor Cells (LEPC) located in the sclerocorneal limbus - mainly the basal area – migrate towards the upper layers of the epithelium while differentiating to renew the tissue. Since their discovery, these progenitor cells have been widely studied and controversial, since definitive biomarkers for identifying LEPCs remain elusive [1]. Alternatively, a combination of putative stemness (ΔNp63α, KRT15, ABCB5, etc.) and differentiation markers (KRT3, KRT12, connexin 43, involucrin, etc.) has been accepted to identify these progenitor cells [2]. Here, our goal was to study the proteomic profile of the corneal cells and determine the expression of stemness-related new biomarkers in the sclerocorneal limbus.

Methods: Six human corneas were dissected to isolate five distinct cell types: limbal epithelium, limbal stroma, conjunctiva, corneal epithelium, and corneal stroma. Each cell type was independently processed for a shotgun proteomic analysis by Liquid Chromatography tandem Mass Spectrometry. Comparative analysis of the resulting proteomes facilitated the identification of differentially expressed proteins within each cell type. A list of 10 candidates for limbal epithelial cell identification was created, using proteomic data and related literature. Finally, PBX Homeobox 1 (*PBX1*) emerged as a candidate for further investigation with additional human corneal samples subjected to multiple immunofluorescence staining. For that, we used different combinations of differentiation markers and proteins that act by forming complexes with PBX1 such as MEIS1.

Results: Inmunofluorescence staining of esclerocorneal sections confirmed the co-expression of PBX1 and KRT15 in the limbus (figure1). A particular expression pattern was observed for PBX1, as nuclear localization was observed on the basal layers of the epithelium while the protein was expressed in the cytoplasm of some suprabasal epithelial cells. No expression of PBX1 was observed in the central area of the cornea, although some presence was observed in the peripheral cornea (data not shown). In addition, MEIS1 expression was observed in the esclerocorneal limbus and peripheral cornea, although no co-localization with PBX1 was obtained.

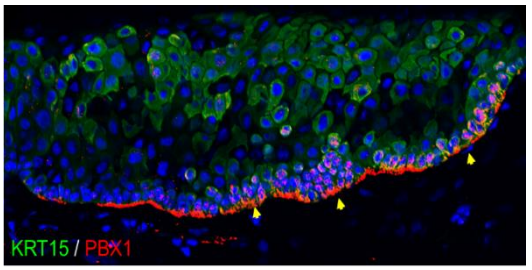

Figure 1. Inmunofluprescence staining of LEPCs in the human esclerocorneal limbus.

Discussion and Conclusion: PBX1 is a transcription factor of the TALE homebox family that acts in complex with MEIS1, which is implicated in multiple cellular processes including development, differentiation, survival and stem cell regulation among others [3]. Nuclear PBX1 expression in the human sclerocorneal limbus implies its potential activity in basal layers, with subsequent cytoplasmic translocation in differentiated cells, possibly diminishing its transcriptional activity. This suggests a potential dynamic regulation of PBX1 transcriptional activity during cellular differentiation. In addition, the expression of MEIS1 in the area where LEPCs are located, suggests that cellular processes related to stem cell regulation and differentiation are present confirming their role in corneal epithelial renewal and regeneration. The authors thank the Department of Heath of the Basque Government (IT524-22 and 2021333007) and the Spanish Ministry (MRA's grant) for funding.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P38 - Presbycusis and Alzheimer's Disease: An Analysis in the New Animal Model APP^{NL-F}

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Introduction: Presbycusis has been associated with a significant increase in the risk of developing Alzheimer's Disease (AD), and it has been observed to accelerate its progression, being considered as the main risk factor in middle age. Despite this epidemiological relationship, the underlying neuropathogenic interactions between both conditions are not yet fully understood. Thisgap in knowledge motivates the investigation of possible mechanistic links between presbycusis and AD, with the potential to improve interventions aimed at delaying, preventing, or reversing presbycusis, and possibly slowing down or halting the progression of AD [1]. The aim of our work is to explore the possible pathophysiological interactions between presbycusis and AD. Specifically, we evaluate the hypothesis that beta-amyloid proteopathy linked to AD in the central nervous system may have effects on the auditory receptor thus, exacerbating preexisting hearing loss and generating a vicious circle between presbycusis and AD.
Methods: We used APP^{NL-F} “knock-in” mice, which carry a "humanized" mutated version of the amyloid precursor protein (APP) gene inserted into their original locus, inducing abnormal APP processing resulting in beta-amyloid deposits formation like those found in human AD, without causing massive overexpression of the protein. We used C57BL/6J mice as a control group, selected from the same strain, and matched by age (6-8 months, and 12-14 months). Brainstem auditory evoked potentials recordings were performed to evaluate auditory function, and morphological analyses of the spiral ganglion were carried out, along with immunoperoxidase testson histological sections of the cochlea to detect the presence of antioxidant enzymes as well as oxidative stress markers.
Results: Functionally, an increase in auditory thresholds at 6 months was evidenced in APP^{NL-F} mice, surpassing the control age-matched C57BL/6J, who naturally develop spontaneous presbycusis after that age. However, these thresholds in both genotypes were similar between 12-14 months. Morphologically, in the 6 to 8 months group, there was loss of spiral ganglion neuronsin the cochlear basal turn of APP^{NL-F} mice compared to C57BL/6J. In the 12 to 14 months group ofboth genotypes, this loss was evident not only in the basal turn but also in the intermediate. Immunoreactivity for GPX-1 and CAT decreased in the cochlea compared to WT mice, with a particularly notable decrease in the stria vascularis, spiral ligament, spiral limbus, and spiral ganglion neurons. These findings, along with the marked increase in the oxidative stress immunomarker 3-NT in APP^{NL-F} mice, point to a significant increase in oxidative stress in the auditory receptor.
Discussion and conclusions: The results demonstrate a significant alteration in the auditorystructure and function in APP^{NL-F} mice compared to age-matched wild-type C57BL/6J mice, supporting the hypothesis that AD proteopathy may contribute to exacerbating presbycusis. These findings suggest a possible connection between both phenomena, which could have significant implications during AD. Therefore, if AD proteopathy affects the peripheral auditory receptor, exacerbating preexisting presbycusis, this could in turn negatively influence the course of AD, leading to a vicious circle of relevant practical consequences.

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P39 - Angiotensin-converting enzyme 2 (ACE2) gene expression in a longitudinal C2C12 sarcopenia *in vitro* model

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Introduction: Sarcopenia is a musculoskeletal disease related to muscle mass and function. Due to its increasing prevalence, identifying early molecular biomarkers subjacent to sarcopenia is mandatory. Angiotensin-converting enzyme 2 (ACE2) is one of the main components of the renin-angiotensin system (RAS) [1]. Imbalanced RAS has been associated with increased cardiovascular risk as well as with muscle-related pathologies such as sarcopenia. This study aimed to generate a three-stage *in vitro* sarcopenia model with C2C12 murine cells: NS (no- sarcopenia), S (sarcopenia), and SS (severe sarcopenia). Afterward, we aimed to characterize the sarcopenia model visually as well as analyze and compare the gene expression of ACE2 along the three-stage model.

Methods: This study has been approved by the Biological Agents Committee of the University of the Basque Country (CEIAB: M30-2021-239). Thesarcopenia *in vitro* model was generated by slighting modification of the protocol [2]. We have phenotypically characterized this sarcopenia *in vitro* model through three different stages corresponding to higher culture passages. The ACE2 mRNA was quantified by Real-Time PCR, normalizing with housekeeping genes GAPDH and RNA18S, calculated as ΔCt. The experiments were performed in triplicate and the gene expression results were relativized with respect tothe values obtained for the NS phase and shown as the mean±SD. A repeated-measures Student's t-test compared means.

Results: Figure 1 shows that the cells adopted a senesce appearance as sarcopenia cells, divided less and their cytoplasm arose full of vesicles(secretory phenotype). Relative gene expression of ACE2 along the aged cell model increases in the S phase and later, and it decreases in the SSphase (Figure 2).

Figure 1: Images obtained by 20x objective microscopy of murine myoblasts. **A:** No-sarcopenia cells, **B:** Sarcopenia cells, **C:** Severe sarcopenia cells.

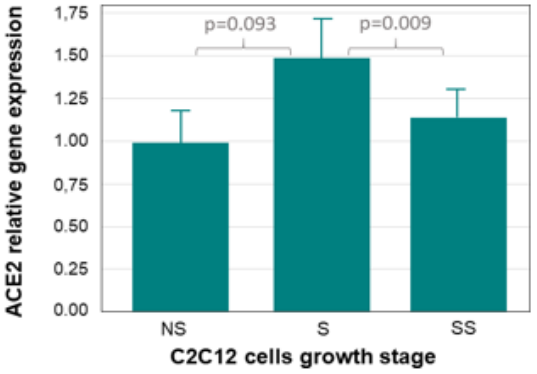

Figure 2: Angiotensin-converting enzyme 2 (ACE2) relative gene expression of C2C12 cells. **NS:** No-sarcopenia cells, **S:** Sarcopenia cells, **SS:** Severe sarcopenia cells.

Discussion & Conclusion: This *anti-U-shape* profile may reflect contraregulatory mechanisms along sarcopenia development. Further research should be developed to identify these mechanisms and clarify the potential role of ACE2 as a biomarker for sarcopenia.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P40 - The deleterious effect of serum from ST-segment elevation myocardial infarction patients on endothelium viability is involved inchanges in cardiac structure

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Introduction: Clinical and experimental studies pointed out abnormalities on the endothelial monolayer in reperfused ST-segment elevation myocardial infarction (STEMI) patients are initiated during ischemia but abruptly boost upon restoration of blood perfusion into the ischemic area. Weaimed to evaluate the effect of serum isolated after revascularization from STEMI patients on the grade of endothelial permeability in vitro, by promoting endothelial cell apoptosis and necrosis in vitro. The association between the percentage of serum-induced endothelial cell apoptosis or necrosis in vitro and the extension of cardiovascular magnetic resonance (CMR)-derived parameters of reperfusion injury (edema, hemorrhage, and microvascular obstruction) was also investigated.
Methods: Human coronary artery endothelial cells (HCAEC) were incubated with serum isolated 24h post-revascularization from 43 STEMI patients submitted to CMR and 14 control subjects. Theeffect of STEMI serum on activation of apoptosis and necrosis as well as on permeability and structure of the endothelial monolayer was assessed using morphometric, molecular and genetic approaches.
Results: Serum isolated 24h post-reperfusion from STEMI patients had a deleterious effect on endothelial cells by activating apoptosis and necrosis in vitro via flow cytometry analysis and TUNELstaining. Post-reperfusion serum also produced a deleterious effect in terms of boosting vascular permeability by enlarging intercellular spaces. Patients whose serum induced a high percentage of necrotic cells in flow cytometry analysis also displayed a more permeable endothelial barrier using an in vitro vascular permeability assay ($p<0.05$) and more compromised CMR-derived cardiac structure at both acute and chronic phases after myocardial infarction.
Discussion & conclusions: Post-reperfusion serum activates necrosis and apoptosis in endothelialcells and increases the grade of endothelial permeability in vitro. The more potent the necrosis- triggering effect of serum, the more deleterious resulting cardiac structure. These data reveal that activation of necrosis, a form of irreversible cell injury characterized by cell membrane rupture, could also participate in promoting microvascular injury, resulting in a more compromised cardiac structure after STEMI.

P41 - Coral health analysis in the face of climate change

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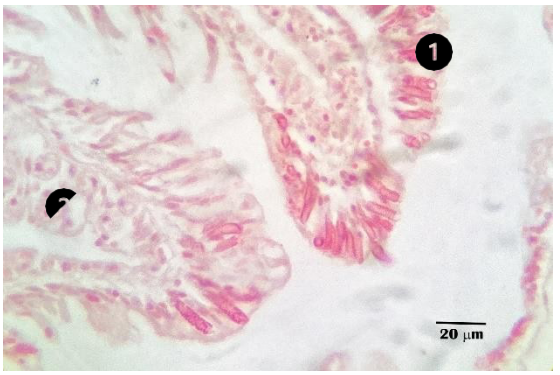
Introduction: In our country, different restoration programs are currently being developed. Coral reefs, in which different institutions participate, however, these efforts will be useless if the transplanted corals cannot withstand warmer oceans; a first step is to evaluate the health of the corals used in restoration areas for the search for biomarkers that allow us to use more resistant corals, therefore which the objective of this work is to determine the health status of two species ofcorals (*Acropora palmata* and *Acropora cervicornis*) in two areas (Quintana Roo and Veracruz, Mexico).

Methods: Coral fragments (*A. cervicornis* and *A. palmata*) were collected in the town of Xcalak, Quintana Roo, and Veracruz, Mexico, by free diving. The samples were fixed in 17% formalin anddecalcified with 10% nitric acid (HNO3) to perform the pathological analysis with a hematoxylin-eosin stain. For the proteomic analysis, total protein extraction was carried out from the bleached coralsand the unbleached corals, and they were analyzedwith mass spectrometry techniques.

Fig.1. spirocytes (1) and zooxanthellae (2) in corals



Results: After carrying out the histological routine in each of the coral samples *A. cervicornis* and *A. palmata*, the main tissue structures of these organisms were observed. Both organism species present epithelial tissue, mesoglea and gastrodermis, as well as spirocytes and zooxanthellae (Fig.1.). When comparing sections of unbleached and bleached *A. cervicornis* and *A. palmata* corals, the decrease in zooxanthellae present in the tissue is observed. The bleached corals also showed the presence of possible parasites in both species and granular cells with melanin.

Discussion & conclusions: Histopathological analysis of samples of *A. cervicornis* and *A. palmata* revealed differences between bleached and non-bleached corals, such as the degeneration of mesenteries which shows that the coral is losing its ability to extract nutrients fromits food [1], melanin granular cells, which, being healing and defense cells, show that the coral is responding to the stress it has suffered [2]. The presence of possible parasites also shows possiblepredation stress. The decrease in zooxanthellae is a more noticeable difference in both species, which is why this has become the most important indicator to show the stress of corals, since whencorals lose these organisms, they also lose energy for all their processes [1]. Bleached and non- bleached corals showed differences such as: decrease in zooxanthellae, presence of possible parasites in both species of bleached coral, and granular cells with melanin.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P42 - Efficacy of cell therapy in the treatment of diabetic foot ulcers. A systematic review

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Introduction: Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia that can lead to chronic complications favoring histologic lesions such as diabetic foot ulcers (DFU) resulting from diabetic neuropathy and vasculopathy. Failed DFUDFU treatments trigger up to 20% of amputations, increasing morbidity and impairing the quality of life of diabetic patients. Hyperglycemic states increase oxidative stress and decrease the synthesis and release of growth factors, which decisively affect wound healing. This makes it necessary to look for new therapeutic alternatives as biological treatments. Cell therapy allows for tissue repair after transplantation of cell populations that can self-renew and give rise to various kinds of functional mature cells. The pluripotency of stem cells and platelet-rich plasma (PRP) makes them potential candidates for accelerating the tissue repair processes of DFU by secretion of cytokines, chemokines, and growth factors. The aim was to critically review clinical trials evaluating the efficacy of autologous cell therapy administered intralesionally injections or topically with PRP, bone marrow-derived mesenchymal stem cells (BM-MSCs), or bone marrow-derived mononuclearcells (BM-MNCs) in the treatment of DFU requiring complex care or unresponsive to conventionaltreatment.

Methods: This systematic review was conducted following the specific recommendations and methodological guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, the PICOS question model, and the McMastercritical review form used for methodological quality assessment. The search was performed in SCOPUS, Web of Science (WOS), Nursing Care (CUIDEN), and Medline (PubMed) databases fororiginal studies published in the last 5 years. The study was registered in PROSPERO (#CRD 42024537847).

Results: Out of the 107 records identified in the search, 5 (1-5) studies met the inclusion criteria, and the methodological quality was between 75% and 87.5%. Two randomized controlled trials (3,5), and 3 prospective studies (1,2,4) were included. Three studies used PRP (2,4,5) and two studies applied BM-MSCs (1,3) or BM-MNCs (3). 71 patients with DM were included, DFU treated with PRP (4) administered topically as a gel or gel plus intralesional injectionachieved a significant ($p < 0.05$) reduction in wound area. The processes of epithelialization (1,2,4,5), or healing (1,4,5) had non-significant ($p > 0.05$) increases after the use of PRP (2,4,5) orBM-MSCs (1) cell therapy. The overall cure rate was significantly improved ($p < 0.05$) over the control group (CG) after the application of either BM-MSCs or BM-MNCs (3). Treatment with eitherBM-MSCs or BM-MNCs was able to non-significantly ($p > 0.05$) reduce the amputation rate (3) concerning the CG. No serious adverse effects were reported (1-3.5).

Discussion & Conclusion:Cell therapy induces natural healing responses through growth factors by promoting cell differentiation and proliferation stimulating the formation of new cells without serious adverse effects. Our findings may indicate that cell therapy with BM-MSCs or BM-MNCs and PRP on non-healing DFUs is a safe and effective therapeutic option in terms of DFU duration, wound healing and epithelialization, and amputation prevention than standard treatment.

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P43 - Cellular death of Leydig cells by necroptosis in hamster and boar testes

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Introduction: Leydig cell loss can be attributed to several factors, but the underlying cellular mechanisms remain poorly understood, under both normal and pathological conditions. Apoptosis has been proposed as a potential method of cell death in some cases. However, in physiological conditions in the Syrian hamster, both in fertile adults and during regression induced by short photoperiod, Leydig cell loss has been associated with the presence of cells exhibiting signs of cellular deterioration in the testicular interstitium. Ultrastructural analysis of Leydig cells has revealed that these cells have necrotic characteristics, suggesting that necroptosis may be the mechanism of their elimination. Similar observations of necrotic Leydig cells have been reported in both normal and cryptorchid pig testes.
Aim: The aim of this study was to verify by immunohistochemistry the presence of necroptosis in Leydig cells of two mammalian species, the Syrian hamster and the boar under normal conditions and in cryptorchid boars.
Methodology: For this purpose, six testes from 6-month-old Syrian hamsters and three from postpubertal boars (*Sus domesticus*) were used. Testicular samples were fixed in methacarn (methanol:chloroform:acetic acid, 6:3:1) and 4% formaldehyde, dehydrated through a graded ethanol series, and embedded in paraffin wax at 56-58°C. Subsequently, 5 µm thick sections were obtained using a rotary microtome (Leica Biosystems) for subsequent analyses. These sections were processed for both histological evaluation with hematoxylin and eosin (H&E) staining and immunohistochemical analysis using antibodies specific to the necroptosis marker RIPK3 (ProSci. San Diego, CA).
Results: Leydig cells exhibiting abnormal characteristics were observed in the interstitium of the examined testes. These cells were found to be positive for RIPK3. In boar cryptorchid testes, the frequency of abnormal cells was apparently higher, particularly in the vicinity of seminiferous tubules with significant spermatogenesis impairment.
Conclusions: The immunohistochemical analysis has confirmed that Leydig cells undergo a necrotic cell death process, which is likely to contribute to their elimination under both normal and pathological conditions.

P44 - Vimentin expression is increased in Sertoli cells of seminiferous tubules' portions with greatest epithelial deterioration in aged testes

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Introduction: Sertoli cells in the seminiferous epithelium are responsible for maintaining both its structure and the viability of germ cells. Vimentin, an intermediate filament within the cytoskeleton, is involved in many cellular functions. In some cases, alterations in the seminiferous epithelium may be associated with changes in the expression of vimentin in the Sertoli cell, affecting both the amount and distribution of this protein. With aging of the seminiferous epithelium, there is a gradual decrease in the number of germ cells, indicating a decline in Sertoli cell function, especially in the parts of the tubules where spermatogenesis is arrested.
Objectives: to evaluate whether the expression of vimentin in Sertoli cells changes in aged seminiferous tubules that show arrest of spermatogenesis.
Methodology: For this purpose, six testes of 24-month-old Syrian hamsters were used. The samples were processed for light microscopy study and immunohistochemistry for vimentin was performed. A semiquantitative study of immunoreactivity was performed. The area of vimentin immunoreactivity (AvR) and the total area of the seminiferous tubules (AST) were calculated, and the AvR/AST ratio was obtained for each type of histological section studied: Type 1: complete spermatogenesis, Type 2: arrest of spermatogenesis in round spermatids, and Type 3: arrest of spermatogenesis in spermatocytes and spermatogonia.
Results: Sertoli cells showed positive vimentin immunoreactivity in the perinuclear zone and cytoplasmic processes in type 1-2 seminiferous tubule sections. In type 3 Sertoli cells, immunoreactivity was stronger around the nucleus and in the basal region of the cell, with decreasing intensity in the processes. The AvR/AST ratio remained statistically unchanged between type 1 and type 2 tubules, although a slight increase was observed. However, a significant increase ($p<0.05$) in the ratio was observed when compared to type 3 (Fig. 1). Sertoli cell desquamation was observed in type 3 tubules with an average of 1,99 vimentin-positive cells in the tubular lumen compared to 0,08 in type 1 and 2.

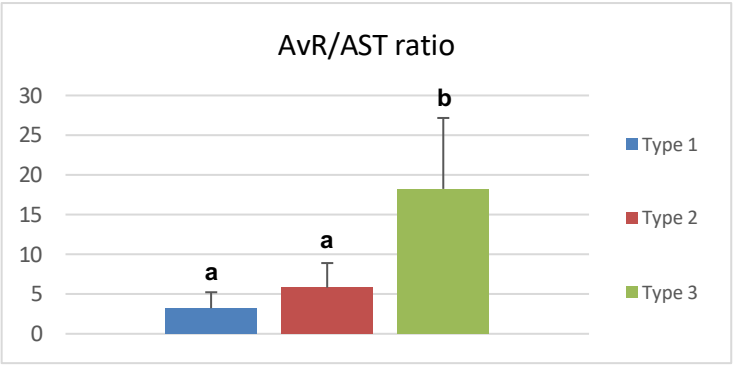

Fig.1. Immunoreactivity of vimentin in three types of seminiferous tubules of aged testes.
[a,b] are significant differences when p value < 0,05

Conclusions: In aged Syrian hamster testes, Sertoli cells exhibit an increase in vimentin expression with a corresponding shift in its cellular distribution. This phenomenon is specifically observed in regions of the seminiferous tubules where spermatogenesis is significantly impaired, suggesting a possible link between altered vimentin expression and Sertoli cell dysfunction and seminiferous epithelial deterioration.

P45 - Leydig cells of intratesticular pathways interstitium of Syrian hamster

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Introduction: In intratesticular pathways, several areas can be differentiated: the final portion of seminiferous tubule, the valve, a transition zone, the straight tubule, and the testicular rete. The histological structure of each area with respect to the epithelial lining is known and has been previously described by some authors. In these studies, it has been suggested that in the interstitium of these areas the cell density of the Leydig cell population seemed greater than in other locations of the testis. Likewise, it has recently been observed that these cells are more resistant to agents that damage them. Objective: consequently, with the above we intend in this communication to determine the morphological characteristics of these Leydig cells and their differences with those of the rest of testes in the Syrian hamster. Along with this, the density of Leydig cells in interstitium of these areas will be evaluated with respect to interstitium that there is between the seminiferous tubules.
Material and Methods: For this we have carried out a qualitative and quantitative study (with light microscopy) in 12 sections of testis stained with H&E both in the parenchyma of the intratesticular pathways interstitium (zone A) and in the normal testicular interstitium (zone B) from 6 six-month-old hamsters. For electron microscopy, the testicular pieces were processed conventionally and the semithin sections obtained were also used for the study with light microscopy. Results: In zone A, different cell types appear, such as fibroblasts, macrophages and many Leydig cells that form groups that reach the wall of the first cisterns of the rete testis. Ultrastructurally, they present abundant mitochondria and a highly developed smooth endoplasmic reticulum. Lipid vacuoles and folding of the smooth reticulum are also observed. An increase in the density of Leydig cells was observed in zone A compared to zone B, since the number of Leydig cells per interstitial volume was significantly higher in A than in B ($p<0.05$).
Conclusions: in intratesticular pathways interstitium of the Syrian hamster there is an accumulation or reserve of Leydig cells that constitute an important part of the total set of them in the testis. Furthermore, these cells show morphological characteristics related to intense functional activity. These results raise the need that in the studies carried out to assess the activity of Leydig cells in the testis, this intratesticular pathways interstitium must be taken into given the high number of them and possibly due to their specific properties.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P46 - Proliferation, apoptosis, and number of Sertoli cell in aging Syrian hamster testes (*Mesocricetus auratus*)

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Introduction: The Sertoli cell (Sc) is key in spermatogenesis, so important that the daily number of sperm depends directly on the number of Sertoli cells in the testes. During aging a decrease in the Sc population has been found in some mammals, which is reflected in a decrease in the quantity and quality of sperm. A renewal rate of the Sertoli cell has recently been described in adult animals and under normal physiological conditions, but no data are available on the aging of this cell.
Objectives: this study aims to determine, on the one hand, whether there is a decrease in Sc during testicular aging. On the other hand, whether this decrease depends on an increase in apoptosis or a decrease in proliferation or both phenomena.
Methodology: For this, a total of twenty male hamsters were used into two groups of 12 and 24 months of age, respectively. The testes were fixed in methacar and embedded in paraffin. Histological sections of testes were observed by confocal microscopy to study Sc proliferation (PCNA + vimentin, immunohistochemistry), Sc apoptosis (TUNEL histochemistry and vimentin immunohistochemistry), and conventional light microscopy (H&E). For the total number of Sc was obtained using a point counting method with the H&E sections. In the groups, the proliferation index (PI) and apoptotic index (AI) of Sc were calculated. For the PI or AI of Sc, it was calculated as the number of [PCNA+ or TUNEL +/Vimentin + Sertoli cells/total number of Sertoli cells] x100. The results revealed the existence of PCNA+ or TUNEL+ and vimentin+ cells in all groups, which corresponded to Sc in proliferation or apoptosis, respectively. Results: Regarding the total number of Sc per testis, a significant decrease was observed in 24-month-old hamsters compared to 12-month-old hamsters ($P < 0.05$). The PI of Sc did not vary significantly between the 12-month and the 24-month group, and the AI was higher in the 24-month group ($p<0.05$), especially due to an increase of apoptosis in the portions of seminiferous tubules with arrested spermatogenesis. A 54% of apoptotic SC in 24-month group was found in these tubular portions. The ratio PI/AI was significantly lower in the 24-month group ($p<0.05$).
Conclusions: The population of Sc cells decreases with age. In aged animals there is an increase in apoptosis in these cells, determining a negative imbalance in cell turnover that could be the cause of the decrease in their number with age. This apoptosis is associated with increase of seminiferous tubule portions with arrest spermatogenesis, and this is probably responsible for decrease in the total number of Sc in the testis.

P47 - Haptoglobin is secreted by the epithelial cells of the bovine oviduct

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Background: Haptoglobin (HP) is synthesized in the liver and is known to bind free haemoglobin released from erythrocytes, thereby inhibiting its oxidative activity. Moreover, HP has traditionally been considered as an acute phase protein that acts within the physiological context of an inflammatory response. The presence of HP in the reproductive organs suggests that it plays a role in mammalian reproductive events. Notably, in pigs, HP has been described with important functions in early embryo development (García-Vázquez et al., Sci. Rep., 2021). The aim of this study is to determine the presence of HP in bovine oviduct by means of immunohistochemistry. Methods: Oviducts were collected from bovine females in late luteal phase and divided into two different sections: ampulla and isthmus. These samples were fixed in formalin and embedded in paraffin. Sections of 5 µm thickness were obtained with a microtome. The slides were then deparaffinised in xylene and rehydrated through a descending series of ethanol baths (100%, 96% and 70%). Following antigenic unmasking, two different polyclonal antibodies were tested: rabbit anti-HP (from LifeSpan BioScience) and rabbit anti-human HP (from Creative Diagnostics). Samples were incubated overnight at 4°C with the corresponding antibody at a concentration of 1/50, 1/250, and 1/500. Subsequently, the sections were incubated for 30 minutes at 34°C with a secondary antibody anti-rabbit IgG (Vector Laboratories). The specific staining was revealed using diaminobenzidine, and finally, all the sections were slightly counterstained with Harris's haematoxylin. The slides were scanned using the digital slide scanner Pannoramic MIDI II (3DHistech) and visualized throughout the CaseViewer 2.4 (64-bit version).
Results: Two different anti-HP antibodies were tested, and in both cases, an important signal was observed at the 1/500 concentration of the primary antibody. HP was broadly detected in the ampulla and isthmus, with a major localisation in the epithelium, where both ciliated and non- ciliated cells showed strong staining in the cytoplasm and the outer surface of cells. No signal was found in the control sections incubated with the secondary antibody.
Conclusion: Our findings provide evidence of the presence of HP in epithelial cells of cow oviducts, thereby demonstrating that these cells contribute to the production and subsequent secretion of the protein into the oviductal fluid. Further studies are required to determine the function of this protein in this species.

[1] García-Vázquez FA, Moros-Nicolás C, López-Úbeda R, Rodríguez-Tobón E, Guillén-Martínez A, Ross JW, Luongo C, Matás C, Hernández-Caravaca I, Avilés M, Izquierdo-Rico MJ. (2021) Evidence of haptoglobin in the porcine female genital tract during oestrous cycle and its effect on in vitro embryo production. Sci Rep. 8;11(1):12041.

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P48 - Impact of different ejaculate fractions on porcine uterine histomorphometry

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Porcine ejaculate is characterized by being emitted in clearly differentiated fractions, each with a distinct seminal plasma (SP) content. In artificial insemination (AI), seminal doses prepared with sperm-rich fraction are usually used. Nevertheless, there is a growing trend towards incorporating other fractions, such as the intermediate and post-sperm fractions. It is well- established that the proportion and composition of SP elicits changes in the female reproductive tract, as well as impact embryo development and offspring growth [1-3]. Thus, it is important to investigate the effect of SP on uterine environment. Therefore, this study aims to explore how the different accumulative fractions of the ejaculate influence uterine histomorphometry in porcine species. For this purpose, a total of 20 sows were divided into a non-inseminated group (control-C, n=5); and 3 groups of sows inseminated (AI group) with different accumulative ejaculate fractions : F1, inseminated only with the rich fraction of the ejaculate (n=5); F2, inseminated with F1 + intermediate fraction (n=5); and F3 (whole ejaculate), inseminated with F2 + poor fraction (n=5). At day 6 post-insemination, the sows were sacrificed, and the uterus were collected. The 3 uterine areas evaluated were the following: Region 1, which belongs to the uterine horn close to the oviduct; Region 2, which corresponds to the central zone of the uterine horn; and Region 3, the uterine horn next to the uterine body. The uterine samples were fixed in formalin, processed for paraffin embedding and stained with Hematoxylin&Eosin. The slides were digitized at 0.172 pixel/µm (Pannoramic MIDI II scanner3D Histech®) and the entire section was used to carry out the measurements with the SlideViewer® microscope. From each sample, 5 measurements of glandular depth, 5 of endometrium thickness and 5 of myometrium thickness were made. Statistical analysis (SPSS®) included an ANOVA and non-parametric Kruskal-Wallis test comparing C and AI groups, semen fractions (F1 vs. F2 vs. F3) and uterine regions (Region 1 vs. 2 vs. 3). Significant differences were considered when p-value <0.05. The results showed significant differences between C and AI groups in glandular depth and thickness of the endometrium and myometrium. Glandular depth was greater in the C group for all anatomical regions studied. In region 1, glandular depth was significantly greater in C and F1 than F2 and F3, while in region 2 the groups C, F1 and F3 were greater than F2. The thickness of the endometrium and myometrium in region 1 was significantly lower in F3 compared to the C group, while in regions 2 and 3 the main differences were found in F1, being greater than the other groups. In conclusion, this study represents the first analysis of uterine histomorphometry following AI with accumulative fractions of the ejaculate. It is noteworthy to observe that the histomorphometric characteristics of the female uterine tract are responsive to AI and the presence of various accumulative fractions of SP in the seminal doses, as well as the volume of SP, which is higher in doses prepared from whole ejaculate. Funded by PID2019- 106380RBI00/AEI/10.13039/501100011033.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P49 - Tissular structure of human foetal membranes at term in diverse utero localizations

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Introduction: the histology of human foetal membranes has been known for years. They completely envelop the fetus and undergo various changes during pregnancy. The different tissue layers that compose them have been little characterized in the different areas of the uterus. Objectives: In the present communication we analysed the histological structure of human foetal membranes after eutocic delivery in various parts of the uterus. Methodology: In this study we studied portions of periumbilical (zpFM) and intermediate (ziFM) and rupture zone (zrFM) of ten foetal membranes from eutocic deliveries at term that were processed for light microscopy using conventional techniques in both paraffin and semithin sections. Histochemistry for collagen and carbohydrates, lectin histochemistry, and immunohistochemistry of various proteins were also performed. Results: the usual tissue layers in foetal human membranes were observed in the three locations studied. In zpFM did not present the chorionic layers, neither the cytotrophoblastic nor the decidual layers, always showing this zone a lower positivity with the collagen and carbohydrate techniques. The most novel results correspond to: a) the existence between the cytotrophoblastic layer and the reticular layer of a acellular zone of connective tissue that resembles both in its histochemical characteristics and structure the compact layer of amniotic membrane; b) a greater stained intensity of amniotic compact layer in zrMF to carbohydrates and fibronectin as well as an increase in this location of fibronectin deposits; c) a strong positivity to various lectins in the spongy and reticular layers with abundant deposits of carbohydrates and fibronectin specially in zrMF. Regarding TgFβ2 it was expressed by epithelial and mesenchymal cells and was observed in the extracellular matrix of amniotic compacta layer with greater intensity in the zrMF. It was also expressed in decidual cells and in lower intensity in cytotrophoblastic cells. TGFβ3 showed a slightly different pattern in that there was no reactivity in the extracellular matrix at any location and decidual cells were not positive in the zrMF. Conclusions: The three studied areas of human foetal membranes showed, on the one hand, specific modifications within a similar basic tissue structure. Thus, the extracellular matrix especially in rupture zone showed together with oedema an increase of its carbohydrates that accumulate with fibronectin. The expression of various proteins in the amniotic or decidual epithelial cells was different in diverse localization of FM. On the other hand, we have been able to differentiate a previously undescribed zone in both zrMF and ziMF, which we call the chorionic compacta because it shows very similar characteristics to amniotic compacta layer, and it is continued with the known basal pseudomembrane.

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P50 - Immunolocalization of IZUMO1 and TMEM95 in Syrian hamster (*Mesocricetus auratus*) spermatozoa

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Introduction: Gamete membrane fusion is indeed a crucial step in the fertilization process, ensuring the combination of genetic material from the sperm and the egg to form a zygote. The identification of essential proteins such as IZUMO1 and TMEM95 has provided significant insights into the molecular players involved [1, 2]. However, the precise mechanisms of its action remain an area of active research. Therefore, the aim of this study was to evaluate the presence and localization of IZUMO1 and TMEM95 proteins in the head of Syrian hamster sperm using fluorescence.
Methods: For this purpose, spermatozoa obtained from the caudal region of the epididymis of three Syrian hamster (*Mesocricetus auratus*) specimens were used and processed to obtain the different physiological conditions: noncapacitated, capacitated and reacted after the induction of the acrosome reaction (AR). Capacitation was performed with modified TALP medium for four hours at 37ºC and 5% CO_2 and RA induction by adding calcium ionophore A23183 for one hour at 37ºC and 5% CO_2. Subsequently, simultaneous immunolabeling was performed to detect both the localization of IZUMO1 or TMEM95 and the acrosomal integrity using the lectin PSA (*Pisum sativum agglutinin*) in hamster sperm. Negative controls were performed. Finally, all samples were visualized under a fluorescent microscope, quantifying the sperm according to their acrosomal integrity and the presence of IZUMO1 or TMEM95.
Results: In general terms, five localization patterns were obtained in relation to the IZUMO1 and TMEM95 proteins: P1 (acrosome), P2 (acrosome and equatorial segment), P3 (equatorial segment), P4 (tip) and P5 (no label). The results showed that the majority patterns in intact spermatozoa in the three conditions were P1 and P2 while in reacted spermatozoa it was P3 and P5. In the case of TMEM95, those sperm that had reacted presented the protein mostly in the equatorial region (P3) regardless of the physiological condition. For its part, immunolabeling with IZUMO1 revealed that only spermatozoa that reacted spontaneously after capacitation retained the protein in the equatorial region, since once the acrosomal reaction was induced, the majority pattern was the absence of labeling (P5).
Discussion and conclusions: Our results reveal the presence of IZUMO1 and TMEM95 in the sperm of the Syrian hamster, which may play a prominent role in the fertilization process of this species. Furthermore, the localization of these proteins has been identified depending on the physiological condition and the acrosomal state of the sperm, highlighting the relocalization of the proteins after the acrosomal reaction. In this way, the presence of IZUMO1 and TMEM95 in the equatorial region after the acrosome reaction could be of great importance for the success of fertilization. Future studies involving the generation of knockouts are necessary to understand the role of these proteins during fertilization.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P51 - Study of the migration of PLCz protein in human sperm depending on the physiological condition

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Introduction: Phospholipase C Zeta 1 (PLCZ1) is an isoenzyme predominantly localized in the head of spermatozoa, playing a crucial role in mammalian fertilization by facilitating oocyte activation and subsequently embryogenesis. Following the fusion of the sperm and the oocyte, PLCZ1 is released from the sperm head into the oocyte cytoplasm. Once in the oocyte cytoplasm, PLCZ1 induces the hydrolysis of its substrate, phosphatidylinositol 4,5-bisphosphate, generating inositol trisphosphate, which in turn triggers the release of intracellular calcium, the primary driver of oocyte activation initiation [1]. Due to the importance of this biomarker in the fertilization process, in this research, we analyzed the presence and localization of the PLCZ1 biomarker in spermatozoa from normozoospermic patients, exposed to different physiological conditions and as a function of the progression of the acrosome reaction (AR). Methods: A total of 5 seminal samples from normozoospermic donors were collected and subsequently processed at the Reproduction Laboratory of the Department of Biotechnology, University of Alicante (Alicante, Spain). For each sample, a seminogram was conducted and 5 different physiological conditions were obtained: noncapacitated (NC), Capacitated for 1 hour (C1), Capacitated for 4 hours (C4), AR induced after capacitation for 1 hour (R1), and for 4 hours (R4). Sperm capacitation was performed using the swim-up method in human tubal fluid medium supplemented with 5 mg/mL bovine serum albumin (BSA) at 37°C and 5.5% CO_2. The induction of the AR was carried out with 0.01 mmol/L Ca^{2+} ionophore and 2 mmol/L calcium chloride for 1 hour at 37°C and 5.5% CO_2. Then, the sperm cells were incubated with a primary antibody PLCZ1 (1:100) produced in rabbit overnight. Afterwards, the cells were incubated with a secondary anti-rabbit antibody conjugated with Cyanine™3 (1:100) for 60 minutes. Subsequently, the samples were incubated for 1 hour with FITC-conjugated lectin (PSA-FITC) at a concentration of 100 µg/mL. Negative controls were performed. For chromatin staining, we used 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI). Finally, the sperm were analyzed using a Zeiss LSM 800 Confocal Laser Scanning Microscope, and statistical analysis was performed using IBM SPSS Statistics 19.0. Results: Regarding the localization of PLCZ1 in human spermatozoa, 6 fluorescence patterns have been identified: P1, without label; P2, acrosome region; P3, acrosome and equatorial band; P4, acrosome and the lateral regions of equatorial band; P5, only in the lateral regions of equatorial band; P6, equatorial band. Our results showed that acrosomal integrity significantly influenced PLCZ1 migration. Specifically, in intact NC spermatozoa, the predominant patterns were P2 and P4. After capacitation at one and four hours (C1 and C4), PLCZ1 migrated to the equatorial band (P3), where it remained in those spermatozoa that once reacted maintained the acrosomal band (P6 and P5). Finally, once the sperm completed the acrosome reaction (R1 and R4), the predominant pattern of PLCZ1 was P1, which is associated with the absence of fluorescence. Discussion & conclusions: The immunolabeling techniques used in this study demonstrated accurate simultaneous visualization of the localization of PLCZ1 and the acrosomal state in human spermatozoa from normozoospermic donors. In addition, our study showed that acrosomal integrity influences PLCZ1 migration, resulting in different localization patterns depending on the physiological condition. It should be noted that the conservation of PLCZ1 in the region of the equatorial segment or on the laterals once the acrosome reaction has occurred is of high biological importance in triggering oocyte activation. Overall, our results contribute to increasing knowledge about the role of the sperm protein PLCZ1 in the fertilization process in humans.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P52 - Immunolocalization of Sperm Adhesion Molecule 1 (SPAM1) during in vitro capacitation and acrosome reaction in rabbit sperm

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Introduction: Sperm Adhesion Molecule 1 (SPAM1) is involved in the egg fertilization process, specifically it is a hyaluronidase implicated in the dispersion of the cumulus-oocyte matrix. This protein is widely conserved in all mammalian species. Although SPAM1 has been reported to be the major testicular hyaluronidase, it has also been shown to be synthesized in the epididymis epithelium. SPAM1 participates in various events, with its role in the dispersal of the cumulus oophorus being crucial due to its hyaluronidase activity at neutral pH. Nevertheless, hyaluronidases perform other functions besides the catalysis of hyaluronic acid, being multifunctional proteins. SPAM1 functions include secondary binding to the zona pellucida (ZP) after the acrosome reaction (ZP domain); acrosomal exocytosis associated with Ca^{2+} signaling mediated by the HA-binding receptor (HA domain); and acidic hyaluronidase activity (AH domain) generated after the acrosome reaction, which may be involved in penetrating the zona pellucida and the perivitelline space in acrosome-reacted sperm.
Methods: A total of seven seminal samples were collected from rabbits from the animal facility of the University of Murcia. The samples were divided to analyze various physiological conditions: non-capacitated sperm (NCS), sperm capacitated for one hour using the *swim-up* technique (CS), and sperm after the induction of the acrosome reaction next to capacitation (ARS). To examine SPAM1 localization in both acrosome-intact and acrosome-reacted cells, a costaining was performed. The sperm cells were incubated overnight at 4 °C with an anti-SPAM1 antibody (1:100) produced in rabbit. Subsequently, samples were incubated for 1 hour in darkness with a Cy3-conjugated secondary anti-rabbit antibody. This was followed by incubation with the lectin PNA-FITC. Finally, the smears were assembled using Fluoroshield™ with DAPI. Proper negative control experiments were conducted. For each condition, two hundred cells were examined using a Zeiss LSM 800 Confocal Laser Scanning Microscope. SPAM1 labeling was evaluated in acrosome-intact sperm cells from NCS and CS, as well as in acrosome-reacted cells from ARS.
Results: SPAM1 distribution was examined in rabbit sperm and revealed four distinct staining patterns. Two staining patterns were found in acrosome intact spermatozoa belonging to NCS and CS conditions. The first one consisted of SPAM1 labeling through the pre-equatorial region (P1), while in P2 an intense labelling in the pre-equatorial region and a faint staining in the post-equatorial region was seen. P1 was significantly higher in both physiological conditions (79.11 ± 33.06 %, p=0.006 in NCS; and 85.74 ± 28.65 %, p=0.001). Two different staining patterns were found in ARS (P3 and P4). P3 was characterized by an absence of labeling, while in P4 SPAM1 labeled the post-equatorial region. Given what has been said, both patterns were present in the acrosome reacted sperm population, being none of them significantly higher (60.15 ± 34.30 % and 39.85 ± 34.30 %, respectively; p=0.290).
Discussion & conclusions: As the main sperm protein with hyaluronidase activity, SPAM1 is essential for crossing the cumulus layer to reach the oocyte zona pellucida. The staining localization of SPAM1 in NCS and CS cells may be explained by this fact. According to some authors, SPAM1 plays an important role in the secondary junction involved in sperm-zona pellucida binding. We observed, however, that SPAM1 was relocalized to the post-equatorial region (P4), but it also seems to disappear after acrosome reaction (P3). In conclusion, our results provide novel molecular insights into SPAM1 protein localization during sperm capacitation and its relocalization after the acrosome reaction in rabbit sperm, which could contribute to boost sperm selection techniques to enhance rabbit production.

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P53 - Immunolocalization of IZUMO1 and TMEM95 in human spermatozoa

Robles-Gómez L.[1], Botella-Miguel, A. [1], Hernández-Falcó M.[1], López-Botella A.[1], Díaz-Fuster L.[1], Ferrer-Cortés N.[1], Sáez-Espinosa P.[1], Gómez-Torres M.J.[1]

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Introduction: Mammalian fertilization is mediated by key sperm fusion and adhesion proteins. Thereby, the sperm proteins IZUMO1 and TMEM95 have been implicated in gamete fusion during mammalian fertilization. However, the topographic location of these proteins and its redistribution during important physiological events remains controversial [1]. The aim of this study was to characterize IZUMO1 and TMEM95 in human spermatozoa during *in vitro* capacitation and acrosome reaction. Methods: Semen samples (n=3) were obtained from normozoospermic donors after written informed consent. The study was approved by the Ethics Committee of the University of Alicante (Alicante, Spain) following the Declaration of Helsinki principles. Once normozoospermia have been proven, samples were divided into five physiological conditions: non-capacitated (NC), one-hour-capacitated (CAP1), four-hour-capacitated (CAP4), acrosome-reacted after one-hour of capacitation (AR1) and acrosome-reacted after four-hours of capacitation (AR4). Capacitated spermatozoa (CAP1 and CAP4) were obtained after the incubation in human tubal fluid (HTF) medium supplemented with albumin using the swim-up technique for one and four hours, respectively. The acrosome reaction was induced by incubation with the ionophore A23187. Sperm samples were incubated overnight (4ºC) with a primary anti-IZUMO antibody (1:100) produced in rabbit. Subsequently, the cells were incubated with the secondary antibody anti-rabbit conjugated with Cyanine™3 (1:100). Then, the samples were mounted with Vectashield® and 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI). In addition, similar protocol was used to characterize TMEM95 at the physiological stages mentioned above. A primary anti-TMEM95 antibody (1:300), also produced in rabbit, was used as a differentiator. The appropriate negative controls served to confirm the specificity of each antibody. Finally, IZUMO1 and TMEM95 location patterns were evaluated in NC, CAP1, CAP4, AR1 and AR4 using Confocal Laser Scanning Zeiss LSM 800 Microscope and statistical analysis was performed by IBM SPSS Statistics19.0. Results: As a result of the microscopical analysis, we described staining patterns common to both IZUMO1 and TMEM95 proteins: acrosomal region (P1), absence of fluorescence (P2), equatorial segment (P3) and acrosomal region and equatorial segment (P4). Firstly, IZUMO1 was present in the acrosomal region (P1) in the 50.33% of the NC tested cells. However, 43.67% of cells at this stage did not fluoresce for IZUMO. In CAP1 and CAP4, the most representative pattern continued to be P1 on the rise (63.00% and 54.67% respectively). This pattern, characterized by fluorescence in the acrosomal region, significantly decreased from CAP1 to AR1 ($p<0.05$), with P2 being the most abundant in AR1 (47.33%) and AR4 (42.00%). For TMEM95, P3 was present in 48,00% of NC spermatozoa, being the most abundant pattern. However, the 45,33% of NC cells did not show fluorescence (P2). After *in vitro* capacitation, the principal pattern was also P3 (59.00% in CAP1 and 73.33% in CAP4) but P2 significantly decreased during the *in vitro* capacitation process up around 20% in both CAP1 and CAP4. After the induction of the acrosome reaction, P3 was the most represented pattern in AR1 and AR4 (56.67% and 60.33% respectively). Discussion & conclusions: The IZUMO1 distribution patterns observed in this investigation are similar to those described during hyaluronic acid selection in human spermatozoa [2]. The similarity of the described patterns between IZUMO1 and TMEM95 suggests a possible interaction between the two proteins during gamete membrane fusion during fertilization. Overall, our results promote the understanding of molecular and cellular basis of gamete fusion.

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Poster Presentations

Session 3

Tissue Engineering
Teaching Innovation in Histology

Histology and Histopathology
From Cell Biology to Tissue Engineering

P54 - Intracarotidally grafted hDPSCs migrate to the infarct zone and contribute to functional neovasculogenesis after ischemic stroke

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Introduction: Human Dental Pulp Stem Cells (hDPSCs) from adult teeth have an extraordinary potential to differentiate to different mature cell lineages. Our group reported and patented a protocol to obtain vascular cells (endothelia and pericytes) when hDPSCs were grown with different formulations of serum-free media compatible with clinical use [1]. Interestingly, hDPSCs were shown to generate fully formed blood vessels, containing both endothelial and mural layers separated by a well distinguishable basal lamina, when grafted to the intact mouse brain. However, this process of brain neovasculogenesis mediated by hDPSCs could also be applied against brain lesions affecting vascular irrigation, such as ischemic stroke.
Methods: hDPSCs were extracted from the dental pulp of tooth pieces of adult healthy donorsand cultured for 1 month in standard media to amplify the cell population in plastic adherent growth conditions. 10 days before the *in vivo* grafts, cells were switched to a vascular differentiation medium containing no fetal serum, which induced the formation of floating dentospheres. The spheres were disaggregated and 500.000 cells were used for intracarotidal grafts in Sprague-Dawley rats that had been subjected to 75 min of middle cerebral artery occlusion (MCAO). Neurological scores were compared between rats that had received hDPSC grafts vs vehicle solution. Two weeks after the grafts, animals were injected with Texas-Red labeled Tomato Lectin, sacrificed by 4% paraformaldehyde perfusion, and their brains post-fixed and embedded to make frozen cryostat sections that were used for immunohistochemistry. Human cells were detected by specific antibodies against human proteins STEM121, hNestin,and hCD31. The integrity of blood vessels was also assessed by Laminin and GFAP immunostaining, and the presence of neuronal progenitors in the brain infarct zone was assessedby DCX labeling. Statistical analysis was performed by IBM SPSS Statistics v28.1.
Results: hDPSCs gave rise to vascular cells only when floating dentospheres were generated in culture. Brain immunostaining analysis at 14 days post-graft showed that hDPSCs had principally migrated to the brain infarct area, in comparison with the contralateral hemisphere. STEM121 labeling showed a clear chemotaxis of hDPSCs to the brain lesion, where they contributed to the formation of new blood vessels, particularly in its periphery. Clusters of hCD31+ cells were also found in the infarct core, but not apparently contributing to blood vessels there. Some STEM121+ isolated cells could also be found in the liver and spleen parenchyma. With regard to the morphology and structural integrity of the hDPSC-generated brain blood vessels, these could reach medium to large sizes, and they showed a clear basal lamina surrounded by the terminal end-feet of astrocytes. Furthermore, these blood vessels were functional and contributing toblood perfusion to the infarct zone, as assessed by their luminal labeling of Tomato Lectin.
Discussion & conclusions: Our findings show that non-genetically modified hDPSCs may be systemically injected and migrate by chemotaxis to sites of brain injury, to contribute to neovasculogenesis and an enhanced neurologic recovery after two weeks. Systemic injection offers good perspectives for the clinical use of these cells, avoiding complicated brain surgery. Despite maximizing brain circulation of hDPSCs by intracarotidal injection, a minority of them reached the liver and spleen parenchyma. Apart for neovasculogenesis, hDPSCs may contribute to the recovery of brain function by other mechanisms like neurotrophic support, immunomodulation, and enhancement of subventricular neurogenesis.
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Histology and Histopathology
From Cell Biology to Tissue Engineering

P55 - Treatment of rabbit VX2 liver cancer by irreversible electroporation

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Irreversible electroporation (IRE) is a method of non-thermal focal tissue ablation that uses electrical currents to irreversibly permeabilize cell membranes. This technique is already being used to treat different tumors such as liver tumors. The aim of the current study is to assess the efficacy of IRE treatment in the rabbit VX2 liver cancer model. A total of 15 New Zealand white rabbits were used in the present study. In 9 animals, 1 mm^3 of VX2tumor was implanted into the middle lobe of the liver. After 9 days of tumor growth, animals were randomly assigned to a tumor control group (n = 6) and an IRE treatment group (n = 6). IRE ablationwas performed on IRE treatment group at 1500 V/cm electric field strength using a versatile high- voltage generator and 2 cm diameter parallel-plate electrodes. One and 8 days after treatment wasperformed, 3 treated and 3 tumor control animals were sacrificed. In addition, the same IRE treatment was performed on 3 non-implanted rabbits (IRE control group) which were sacrificed 19days after treatment. The treated / implanted lobes were collected and fixed in 10% formalin. Procedures were approved by the Animal Experimentation Ethical Commission, University of Zaragoza (permit number: PI35/22). Formalin-fixed tissues were trimmed, the volume of the tumorswas determined and samples were processed according to standard histopathological procedures.In addition, tissue sections were processed for immunohistochemistry using the primary mouse monoclonal antibodies anti-ki67 and anti-p53 (Dako/Agilent, CA, United States). One day after IRE treatment, the cut surface of the IRE-ablated zone was characterized by an irregular mixture of white, discolored, and dark areas. Microscopic evaluation of the IRE-ablated tissue showed areas of coagulative necrosis surrounded by large areas of inflammatory cells and congestion. Immersed in the ablated tissue, sheets of viable tumor cells (Ki-67 and p53 positive) were observed. Eight days after IRE treatment, 2 animals showed a well delineated tumor (volumeof 0.25 and 0.55 cm^3) composed of large sheets of viable tumor cells with necrotic areas of varioussizes. The tumors were surrounded by IRE-ablated liver tissue characterized by the coexistence of areas of necrosis with areas of repair. In one animal, no tumor was observed either macroscopicallyand microscopically in the IRE-ablated liver tissue. In the IRE control samples, treated tissue was loose completely the characteristic liver architecture and was composed of reparative tissue. Under the conditions of the present study, IRE treatment did not completely ablated the VX2 liver tumor. A previous report using multiple IRE applications resulted in complete ablation of the VX2 tumor [1]. Differences in electrodes and applied electric field parameters may account for differences in treatment efficacy. In the present study, parallel plate electrodes were used, which generate a more homogeneous electric field distribution and allow large volumes to be treated morehomogeneously. Differences between the electrical conductivity of tumor and normal liver tissue may also explain treatment failure.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P56 - Effect of Vasoactive Intestinal Peptide on human osteocyte differentiation

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Introduction: Bone remodelling is a dynamic process through which old or damaged bone tissue isreplaced for new tissue thanks to a set of cellular events [1]. The resorptive activity of the osteoclastand the bone matrix synthesis activity of the osteoblast must be coordinated to maintain bone integrity. However, the interaction between osteoclasts and osteoblasts should not be studied juston its own but in conjunction with the influence of osteocytes. This cell type derives from the maturation process of osteoblasts when they are encapsulated by the bone matrix itself and they produce a variety of proteins and factors that modulate osteoclast and osteoblast activity. For this reason, recent research has positioned the osteocyte as the third cell type orchestrating the cross-talk to maintain bone homeostasis [2]. Moreover, the fine-tuning of this dynamic system iscontrolled by an intricate network of paracrine and autocrine factors, including neuropeptides such as vasoactive intestinal peptide (VIP) [3]. In this sense, VIP has shown protection against bone erosion in murine models. Its effects have been confirmed in human cells, inhibiting osteoclastogenesis and promoting osteoblast differentiation *in vitro* [4]. Besides, due to thecomplexity of osteocyte culture, there has been no research to date on the effect of the peptide onhuman osteocyte differentiation. Therefore, the main aim of this study is to elucidate the effect of VIP on the differentiation of primary human osteocytes to gain a better understanding of the mechanisms that mediate the osteoprotective effects of this neuropeptide. Methods: Osteoblasts precursors were isolated from human femoral heads and differentiated into mature osteoblast *in vitro*. Osteocyte differentiation cultures were established by integrating mature osteoblasts into a three-dimensional collagen matrix. These constructs were incubated for 14 daysfor osteocyte differentiation in the presence or absence of 10^{-8}M VIP. Then, the gene expression of specific markers of the osteocytic lineage, such as the late osteoblast marker (*BGLAP*) and mature osteocyte markers (*MEPE* and *SOST*) was analysed. Moreover, RANKL and OPG gene expression, central mediators of the cross-talk between osteoblasts and osteoclasts, was also studied. Finally, actin staining was performed on the osteocytes differentiation gels and visualized afterwards by confocal microscopy. Results: Data from osteocyte gene markers showed a significant decrease in *BGLAP* and an enhanced gene expression of *MEPE* and *SOST* in cells differentiated in the presence of VIP. Thesecells also revealed a significant increase in levels of OPG transcripts resulting in downregulation ofRANKL/OPG ratio. Fluorescence actin staining suggested that cells exhibit a different morphology in VIP presence, related to more mature stages of osteocyte differentiation. Discussion & conclusions: All these results indicate that the presence of VIP might accelerate human osteocyte differentiation and support the potential of this neuropeptide as an osteoprotectivefactor. Therefore, while additional studies are needed to evaluate the suggested osteoinductive effects of VIP, our results provide an initial step in its role as a mediator in bone homeostasis.

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P57 - Characterization of the anti-inflammatory potential and microglial behavior of the conditioned medium of Dental Pulp Stem Cells.

Manero-Roig I.[1,2], Pardo-Rodríguez B.[1], Martín-Colomo S.[3], Luzuriaga J.[1], Salvador- Moya J.[1], Basanta-Torres R.[1], Polo Y.[4], Larrañaga A.[3], Barreda G.[5], Lanore F.[2], Ibarretxe G.[1], Humeau Y.[2] and Pineda JR.[1,6]

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Recent strategies have focused on the potential of stem cells secretome as it can modulate the physiological state even of distant cells while avoiding the major drawbacks and risks of cell therapy. In this work, we analyzed and tested the secretome of Dental Pulp Stem Cells (hDPSCs)as a possible alternative for brain therapy. Firstly, we characterized the inflammatory and lipidomic profile of hDPSCs and the differences between their secretomes depending on the cell culture stimulus, hereafter called conditioned media (CMs) and how CM modulates the state and phagocytic activity of mouse microglia *in vitro*.

hDPSCs were obtained from healthy donors and cultured and expanded during 2-3 weeks. Then, cells were deprived of serum and cultured under different conditions during the last 24 hours beforecollecting the CMs. Conditioned media were classified into basal, pro-inflammatory environment using LPS at 20 μg/mL and hypoxia-stressed limiting the oxygen exchange. RT-qPCR analysis confirmed the increase of TNF- α and HIF-1α under pro-inflammatory and hypoxic conditions of cell pellets. Next, we compared the alterations of lipidomic profiles using membrane microarray andmass spectrometry techniques, detecting changes in 78 lipid species. Hypoxic cultures showed a significant increase with respect to normoxia in phosphatidylethanolamines, phosphatidylglycerols and various plasmalogens and decreases in diacylglycerides. On the other hand, inflammatory environment triggered an increase in all sphingomyelins, hexaceramides and decreases in severaldiacylglyceride ethers, with respect to basal control hDPSCs.

hDPSC supernatant were serially centrifuged to discard cell debris, and concentrated prior to use.We found that hDPSCs secretome released mRNA for immunosuppressant ectonucleotidase CD73 and the anti-inflammatory CD39. To check their potential on brain immunomodulation we cultured BV2 mouse microglial cells with the different CMs. Specifically, we evaluated the capacityof the hDPSCs secretome to change the phagocytic dynamics of microglia over time in normal condition or previously stimulated with LPS (20 μg/mL). For that, we used (800 ng protein/mL) of hDPSCs supernatant and prior to videorecording analysis we added $2 \cdot 10^9$ FluoSpheres to determine BV2 phagocytic dynamics over time observing a decrease of phagocytic activity between6h and 12h post CM addition. On the other hand, we made an ELISA to measure the levels of TNFα produced by microglia under the conditions mentioned as an indirect measure of the state ofactivation of the cells. Our results demonstrate hDPSCs drastically alter the lipidomic profile depending on their culture conditions and their sectretome is a powerful tool to reduce the phagocytic capacity of LPS-stimulated BV2 cells.

This work has been financed by the Basque Government (IT1751-22 and 2023333035), Polimerbio SL (2023.0012) and the University of the Basque Country (UPV/EHU) (COLAB22/07). IMR and SMC obtained a Ph.D. fellowship from University of the Basque Country (UPV/EHU) (PIFBUR21/05 & PIF22/119). BPR, JSMobtained a Ph.D. fellowship from Basque Government (PRE_2023_2_0112 & PRE_2023_2_0038). YP has a Bikiantek PostDoc grant (010-B1/2023).

Histology and Histopathology
From Cell Biology to Tissue Engineering

P58 - Effect of VIP on chondrocyte differentiation and cartilage degeneration in osteoarthritis

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Osteoarthritis (OA) is a rheumatic disease characterized by progressive degradation of articular cartilage, loss of subchondral bone and synovitis. The main cells involved are articular chondrocytes (AC). These cells cultured *in vitro* in monolayer undergo a process of dedifferentiation. Three-dimensional cultures with biomaterials such as alginate are often used to redifferentiate them. During OA, AC produce inflammatory mediators and degradative enzymes, destroying the extracellular matrix (ECM) and releasing fragments with catabolic properties, such as fibronectin fragments (Fn-fs). On the other hand, the anti-inflammatory potential of vasoactive intestinal peptide (VIP) has been described in multiple musculoskeletal pathologies. In this study we set out to investigate the effect of VIP on chondrogenesis from bone marrow mesenchymal stem cells (BM-hMSC) from healthy donors in three-dimensional cell pellet cultures. In addition, we analyzed its modulatory capacity on the production of inflammatory and cartilage ECM destructionmediators, by real-time PCR and ELISA, in AC from OA patients in alginate beads, in the presence of Fn-fs as a proinflammatory stimulus. Our results show that VIP accelerates the process of chondrogenesis from BM-hMSC from healthy donors, inducing a higher expression of chondrogenic genes at day 12 of differentiation compared to day 21 in basal conditions. Conversely, alginate beads allow redifferentiation of AC from OA patients, preserving their viability. In these cultures, VIP reduces the production of C1R, MMP1 and MMP13, corroborating its anti-inflammatory and beneficial potential in the process of degradation and loss of articular cartilage in osteoarthritis.

This research was funded by Fondo de Investigación Sanitaria, Instituto de Salud Carlos III (ISCIII) co- financed by Fondo Europeo de Desarrollo Regional (FEDER), grant numbers PI20/00078 andRD21/0002/0004.

P59 - Citotoxicity assays for human mesenchymal cells in presence of porous alumina-wollastonite-silica gel.

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Introduction: Bone tissue has the capacity to regenerate in response to several injuries in a process largely mediated by human mesenchymal stem cells (hMSCs). During osteoregeneration processes, hMSCs differentiate into osteoblasts, which represent a key role in differentiation step in the bone repair. Studies of bone tissue engineering is increasingly developing new materials compatible that allow bone tissue regeneration. These biomaterials must meet some requirements, such as being bioactive, biocompatible, biodegradable and interact with hMSCs. In this work, we propose the synthesis of porous alumina-wollastonite-silica gel (pAWS gel) as a potential bone scaffold material for bone tissue engineering.

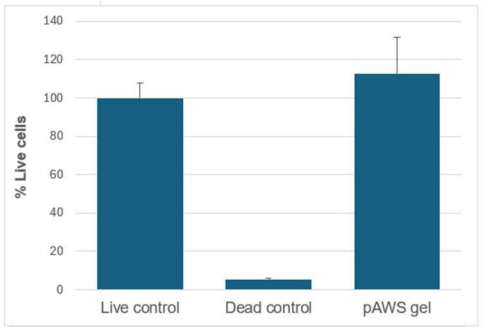

Figure 1: Citotoxicity assay of MSCs after 72 hours in culture, grown in the presence of pAWS.

Methods: We manually mix commercial alumina with 20 wt% wollastonite and 30 wt% carbon for gas chromatography. This mixture of powders is deposited in a graphite mold to later be sintered using SPS (SparkPlasma Sintering) at 1300 ºC and with a uniaxial pressure of 80 MPa. The carbon is then removed by calcination at 900°C for 5 hours and the porous sample is then subjected to a consolidation heat treatment at 1600°C for 2 hours. MSCs were seeded on the pAWS gel under sterile conditions and after 72 hours, analyzed for cell viability and interaction with materials.

Results: Cell survival assays demonstrated that the material studied does not affect the viability of the cells. (Figure 1). Furthermore, using fluorescence microscopy we verified that the hMSCs interact with pAWS gel (Figure 2). These findings indicate that pAWS gel may be apromising material to be used asa scaffold in the treatment of bone diseases.

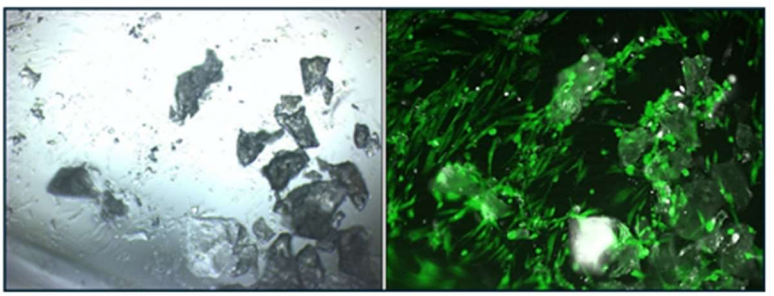

Figure 2: MSCs-pAWS interaction examined with fluorescence microscope (10× objective lens).

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P60 - Adiponectin variations in muscle volumetric loss lesions reconstructed with adipose tissue implantation in rat.

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Introduction: Adiponectin (APN) is a glycoprotein with an anti-inflammatory and promyogenic effect that induces the expression of skeletal muscle-specific markers and induces satellite cell myogenesis. Adiponectin levels are decreased in Duchenne muscular dystrophy, where muscle regenerative capacity is lost. This suggests that APN may be playing a very important role in skeletal muscle regeneration. Considering that APN is primarily secreted by white adipose tissue, our group has developed an experimental model consisting in the implantation of autologous adipose tissue in a volumetric muscle loss (VML) demonstrating the neoformation of skeletal muscle tissue at the site of injury. In this sense, we wondered whether this muscle neoformation may have been favored by an increase in APN as a consequence of the adipose tissue grafting at 7 and 14 days post-implantation.
Methods: 20 male Wistar rats were divided into 5 experimental groups: normal control (CN); regenerative control (CR) with rats that had a myotoxic lesion induced in the tibialis anterior muscle;fibrotic control (CF), which had a VML lesion induced in the TA muscle by a punch; fresh autologoustransplantation (TAF) with rats that were induced with a VML lesion and subsequently implanted with autologous adipose tissue; cryopreserved autologous transplantation (TAC) with rats that were induced with a VML lesion but the autologous adipose tissue underwent a cryopreservation process prior to implantation. To measure adiponectin levels, the ELISA immunoassay technique was used.Histological staining techniques were used to observe morphological differences.
Results: There was an increase in adiponectin levels except in the CF groups. Except for the day 14 TAC group, the CR groups had higher adiponectin levels, whereas the groups with the lowest level were the CF groups. Adiponectin was mostly elevated on day 7 postlesion in all groups except the TAC group on day 14.
Discussion & conclusions: Although there were no statistically significant differences, an increase in adiponectin was observed. The CR samples contained regenerative muscle tissue, whereas in TAF and TAC the samples corresponded to adipose tissue and regenerative muscle tissue, in which APN is known to be produced. This could explain why APN levels were higher in the CR group, where the regenerative process is normal, whereas that happening in the adipose tissue implant groups is not. While APN levels in the CR, CF and TAF groups were highest on day 7 post-injury, which is consistent with a study where APN gene expression was observed to be highest on day 7post-injury, in the TAC group they were highest on day 14. Interestingly, adipogenic markers wereincreased by cryopreservation, as was an increase in cytokines in adipose tissue the longer the cryopreservation time, which may explain this finding.

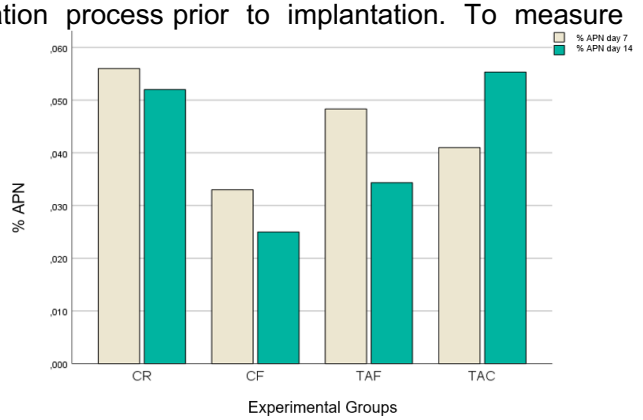

Figure 1. Distribution of APN levels on days 7 and 14 post-injury in the study groups.

Funding: Supported by grant PS-2020-946 from Consejería de Salud y Familias, Junta de Andalucía, Spain.

Histology and Histopathology
From Cell Biology to Tissue Engineering

P61 - Histological evaluation of the innervation in the lesion due to volumetric muscle loss reconstructed by transplantation of autologous adipose tissue

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Introduction: One of the important consequences of volumetric muscle loss (VML) injuries is the effect of secondary denervation due to alteration of neuromuscular junctions. In the present studywe have histologically analyzed whether the transplantation of autologous adipose tissue in the VML lesion reduces the changes linked to denervation and favors the innervation of the newly formed muscle.
Methods: Wistar rats were distributed into 4 experimental groups: normal group (N), VML lesion group, VML lesion group with fresh autologous adipose tissue implant (FTA) and VML lesion with cryopreserved autologous adipose tissue implant (CTA). A histological, histochemical and immunofluorescence study was performed after 60 days of evolution in the preserved area of the edge of the lesion and inside it. The changes linked to the denervation and reinnervation processeswere quantified and statistically analyzed: disseminated neurogenic atrophy, fascicular atrophy, moth-eaten muscle fibers, core-targetoid fibers and groupings of fiber types.
Results: In the VML group, angulated atrophic muscle fibers and cytoarchitectural changes were observed at the edge of the lesion, while the center of the lesion was occupied by fibroadipose tissue. In the TAF and TAC transplant groups, the changes were a reduction in the number of angulated atrophic fibers and a greater number of cytoarchitectural changes indicative of reinnervation. The latter were significantly more abundant when cryopreserved adipose tissue wasimplanted.
Discussion and conclusions: The results show that the transplantation of autologous adipose tissue, both fresh and cryopreserved, reduces the changes linked to secondary denervation in theVML lesion and favors the differentiation of histochemical types in the new muscle tissue generatedin the defect.

Funding: Supported by grant PS-2020-946 from Consejería de Salud y Familias, Junta de Andalucía, Spain

Histology and Histopathology
From Cell Biology to Tissue Engineering

P62 - Histological characterization of post-regeneration sequelae in different injury models

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Introduction: After injury, the presence of apparently normal muscle fibers, but with nuclei in an internalized position, is considered a histological sign that the skeletal muscle has completely regenerated. However, there are other less frequent structural changes that are not considered when evaluating the effectiveness of the regenerative process and that could explain the susceptibility of skeletal muscle to reinjury in physical and sports activity. In the present study we compare the histological and histomorphometric features of rat skeletal muscle in three experimental injury models of different severity.
Methods: Four groups were established, of four Wistar rats each, and the interventions were performed on the two gastrocnemius muscles of each animal: normal rats (N), rats injured by intramuscular injection of a myotoxic agent (MI), rats injured by contusion (MC). and laceration injured (LC) rats. The gastrocnemius muscles were extracted 30 days after injury and processed for microscopic study. The samples were analyzed using histological, histochemical and immunohistochemical techniques. A histomorphometric analysis of the following parameters was performed in representative areas: area occupied by connective tissue, number of muscle fibers, cross-sectional area of fibers, split fibers, fibers with internalized nuclei. The results were evaluatedwith the corresponding statistical analysis.
Results: Our results show that, while the MI group only differed significantly from the N group in thenumber of fibers with internal nuclei and cracked fibers, the MC and LC groups showed significantdifferences in all the parameters analyzed. The features that most characterized the laceration injury were fibrosis and atrophy of the muscle fibers, while in the contusion injury they were crackedfibers. In the MC and LC groups the number of fibers with internalized nuclei and split fibers do notseem to be determined by fibrosis.
Discussion & conclusions: Our results indicate that regenerated muscles have structural sequelaethat differ depending on the type of injury. This could not only limit their functional capacity but could also be the expression of a potential susceptibility to re-injury. These observations may contribute to a better understanding of the mechanisms of recurrent injuries in sports activity.

Funding: Supported by the CTS-985 Muscle Regeneration Group of the University of Córdoba (Spain).

Histology and Histopathology
From Cell Biology to Tissue Engineering

P63 - Bone regeneration promoted by a titanium membrane seeded with adipose tissue-derived mesenchymal stem cells in a critical defect of rat mandible

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Introduction: Bone regeneration capability has limitations due to numerous factors such as type ofbone, zone, size of the defect, age, etc., a concern present in traumatology, orthopedics and odontology. The critical size of bone defect to heal spontaneously has been considered smaller than 4 mm in diameter, therefore larger bone defects require a graft, autologous graft appearing asthe gold standard, but showing several drawbacks such as morbidity, scarce material, infection andincreased surgical interventions. Thus, artificial grafts are being provided by tissue engineering seeking for their osteoinduction, osteoconduction and osteointegration. The materials employed are mainly ceramics, metals and biopolymers, cells and soluble factors also being employed. Among the metallic scaffolds employed, titanium alloys have been widely used because of the high biocompatibility, therefore, we have assessed the study of bone regeneration using a titanium membrane and adipose tissue-derived mesenchymal stem cells (ASCs) in rat mandible.
Methods: Young Wistar rats were divided into 4 groups (n=5): A) Positive control in which a subcritical defect (2-3 mm) was created; B) Negative control having suffered a critical osteotomy (4 mm) with no help to heal; C) Critical osteotomy plus titanium membrane; D) The same as groupC but with autologous ASCs seeded onto the titanium membrane. The titanium membranes appliedwere perforated with near 400 µm pores. One month after surgery, the animals were anesthetized and sacrificed by transcardial perfusion with 4% paraformaldehyde, and studied for light microscopy by means of blue toluidine, alizarin red, Von Kossa and Fluoropaque (1) methods, for scanning and transmission electron microscopy and for energy-dispersive X-ray microanalysis (EDAX). Care and management of animals always followed the guidelines and legislation regardingtheir use in laboratory.
Results: The osteotomy in group A appeared closed by newly formed bone tissue, whereas the animals in group B hardly showed bone regeneration. Animals in groups C and D showed considerable bone regeneration, being more complete in the latter. No significant inflammatory response was observed. The newly formed bone appeared trabecular with active osteoblasts andosteoclasts and showing a composition of the matrix compatible with hydroxyapatite according to EDAX. Adipocytes and striate muscle cells could be seen only in group D.
Discussion: Our study supports the usefulness of titanium membrane in guided bone regenerationas well as the significant role of ASCs added to the graft. The presence of pores in the titanium membrane is also important since it avoids the penetration of soft tissue which interferes in osteogenesis while let the income of nutrients, oxygen, and osteogenic cells. Adipocytes and myocytes found in group D likely derive from differentiation of the ASCs added.
Conclusions.
1. Bone defects less than 4 mm are repaired spontaneously.
2. Bone defects equal or larger than 4 mm are not able to heal without help.
3. A porous titanium membrane grafted to a critical bone defect is well integrated in the host tissueand favors its regeneration to close the osteotomy after one month.
4. ASCs seeded on the titanium membrane graft significantly help bone regeneration.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P64 - Implementation of the BIOCLEFT advanced therapies clinical trial in infants with cleft palate

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Introduction: The surgical management of patients born with cleft palate is challenging, due to thelack of healthy tissues available for an efficient closure of the palate defect. In this regard, the recentdevelopment of tissue engineering allowed the generation in the laboratory of human bioartificial tissues showing promising clinical results, such as the skin and cornea [1]. In the case of cleft palate, our group previously developed a model of human palate mucosa by tissue engineering that showed potential usefulness in laboratory animals. However, the clinical translation of this artificial tissue, considered as an Advanced Therapies Medicinal Product (ATMP) is very complex.Methods: A pioneering ATMP clinical trial called BIOCLEFT has been designed to evaluate the potential biosafety and usefulness of the human palate mucosa generated by tissue engineering by the research group. To obtain approval by the Spanish Medicines Agency (AEMPS), all the preclinical results corresponding to the ex vivo and in vivo evaluation of the bioengineered palate mucosa were analyzed. With these data, we proposed a preclinical assessment to the AEMPS, who evaluated positively the preclinical results, but asked us to carry out additional experiments toevaluate the effects of acellular fibrin-agarose biomaterials in laboratory animals. After these experiments were carried out, authorization was requested to implement an advanced therapies clinical trial in infants with cleft palate.
Results: After analyzing the preclinical results, the AEMPS approved the BIOCLEFT clinical trial (EudraCT number 2023-506913-23-00), that was initiated in 2024. BIOCLEFT has been designedas a phase I-IIa, randomized, controlled, and unblinded clinical trial to evaluate the safety,feasibility, and preliminary efficacy of an autologous human palatal mucosa substitute generated by tissue engineering, in the treatment of cleft palate patients. 5 patients will be initially treated to evaluate biosafety. The gold-standard treatment will be performed (uranostaphylorrhaphy), but thedenudated bone will be covered with a palate mucosa generated by tissue engineering. If the results confirm that that the treatment is safe, 10 additional patients will be enrolled, with 5 of themcorresponding to the control group. Variables considered in the clinical trial protocol include several parameters related to biosafety (biointegration, local and systemic side effects), biomorphometric measurements of maxillofacial and cranial bones and soft tissues, facial growth and development.Discussion & conclusions: Obtaining approval for an advanced therapies clinical trial involving tissue engineering in children and infants is very challenging, as the possible effects of bioengineered tissues have not been determined in these patients. In the present work, we described the steps necessary to implement this type of clinical trials requiring AEMPS authorization. This clinical trial will allow us to evaluate the potential benefits of a palate mucosa generated by tissue engineering for the treatment of cleft palate infants.
Acknowledgements: This work was supported by grants ICI19/00024 (BIOCLEFT) and FIS PI21/00980, funded by Instituto de Salud Carlos III (ISCIII), Ministry of Science, Innovation and Universities (Plan Estatal de Investigación Científica, Técnica y de Innovación 2021-2023, and Plande Recuperación, Transformación y Resiliencia), and co-funded by the European Union, Fondo Europeo de Desarrollo Regional ERDF-FEDER.
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P65 - Functionalization of bilayered human bioengineered tissue models using bioactive molecules derived from virgin olive oil

Ortiz-Arrabal O.[1,2], Martín-Piedra M.A.[1,2], Chato-Astrain J.[1,2], Carriel V.[1,2], Crespo P.V.[1,2], de la Cueva P.[1], Garzón I.[1,2], Mesa-García M.D.[2,3,4], Gómez-Llorente C.[2,3,4], Alaminos M.[1,2]

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Introduction: Severe conditions affecting the human skin and cornea are very difficult to manage, and novel regenerative treatments are in need. In this sense, human tissue substitutes generated by tissue engineering offer a promising alternative showing potential clinical usefulness. However,biofabrication of bioartificial tissues normally requires massive amounts of cultured epithelial cells,which typically have low proliferation rates in culture [1]. For this reason, culture protocols should be optimized with the use of bioactive molecules able to improve cell proliferation. Virgin olive oil isa source of different types of biomolecules with beneficial health properties, and previous studies reported the use of maslinic acid obtained from olive oil to improve keratinocyte cell cultures [2]. However, its effects has not been determined in artificial tissues.
Methods: Human artificial bilayered tissue substitutes were generated using fibrin-agarose biomaterials with human stromal cells immersed within, and an epithelial layer on top. These substitutes were kept *in vitro* for 12 days in absence and in presence of maslinic acid at a concentration of 5 µg/mL. At 24 hours and 4, 8 and 12 days of follow-up, tissue substitutes were fixed in formaldehyde for further characterization. Histological and immunohistochemical analysis of samples were performed using hematoxylin-eosin staining and marker of proliferation KI67, respectively. Epithelial thickness and number of KI67-positive cells were quantified.
45Results: Bioartificial tissues were successfully developed. Histological analysis showed a superficial epithelial layer consisting of several strata resembling a native epithelium, and a subjacent stroma containing stromal cells and biomaterials. Quantification of epithelial layer thickness revealed that this parameter was significantly decreased in artificial skin cultured with maslinic acid as compared to controls, although cells were well-differentiated. However, non-significant differences were found when KI67-positive cells were assessed in both experimental groups (Fig. 1).

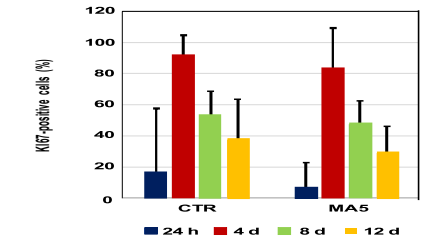

Figure 1. Cuantification of Ki67-positive cells in human bioartificial tissues functionalized with 5µg/mL of maslinic acid (MA5), and control non-functionalized tissues (CTR).

Discussion & conclusions: Our results showed that the structure of human artificial bilayered tissues retained the typical structure of a human native tissue. The number of epithelial cells was not increased with the use of maslinic acid, in contrast with previous studies that showed an improvement of epithelial proliferation in bidimensional cultures. Results suggest that maslinic acid is able to increase epithelial differentiation, but not epithelial proliferation, when used in bioartificial tissues. These results support the use of maslinic acid to promote tissue differentiation in bioengineered models of human cornea and skin generated by tissue engineering.Acknowledgements: This work was supported by grant PE-0395-2019 from Consejería de Salud yConsumo, Junta de Andalucía, Spain. Supported by grants FIS PI23/00335 and ICI21/00010 (NANOULCOR), funded by Instituto de Salud Carlos III (ISCIII), Ministry of Science, Innovation andUniversities (Plan Estatal 2021-2023, and Plan de Recuperación, Transformación y Resiliencia). Supported by grant C-CTS-032-UGR23, Plan Propio de Investigación y Transferencia de la Universidad de Granada 2023, Consejería de Universidad, Investigación e Innovación, Junta de Andalucía. Co-funded by the European Union, Fondo Europeo de Desarrollo Regional ERDF- FEDER, Programa Operativo FEDER Andalucía 2021-2027.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P66 - Histological characterization of NANOULCOR human cornea substitutes generated by tissue engineering

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Introduction: Several diseases can affect the human cornea and cause blindness. Severe cases are treated with cornea transplantation, although this method has some limitations [1,2]. NANOULCOR is a cornea substitute developed by tissue engineering which showed good biocompatibility and functionality in patients with corneal ulcers [3]. In the present work, we carriedout a histological characterization analysis of these corneal substitutes to determine their phenotypic profile and potential mechanisms associated to their positive clinical results.

Methods: NANOULCOR artificial corneas were generated using fibrin-agarose biomaterials containing human keratocytes and limbal epithelial stem cells on top.

These tissue substitutes were cultured for four weeks using the air liquid technique during the last weeks, to promote epithelial differentiation. At the end of the follow-up time, NANOULCOR corneas were fixed and processed for further histological analysis, using the human cornea and the scleral limbus as controls. Hematoxylin-eosin staining, and cytokeratin 15 immunohistochemistry were used, and results were compared between NANOULCOR and control tissues.

Results: Histologically, human artificial corneas showed an epithelial layer consisting of 5-6 cell strata and a subjacent stroma withabundant keratocytes and fibrin-agarose biomaterials. Human native cornea revealed the presence of an epithelial layer with 4-5 cell strata and a well-organized avascular stroma containing numerous collagen lamellae. In turn, the native human scleral limbus displayed the typical limbal morphology, with limbal epithelial stem cells organized in Vogt palisades, and a stroma containing blood vessels. Immunohistochemical analysis of cytokeratin 15 showed the presence of few positive cells in NANOULCOR and native corneas, in contrast with the strong positive signal shown by the native human scleral limbus.

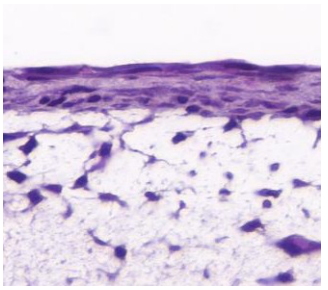

Figure 1. Human artificial cornea generated with fibrin-agarose biomaterial and limbal epithelial stemcells.

Discussion & conclusions: Our findings confirm that the histological structure of NANOULCOR artificial corneas resembled the typical structure of the native central cornea, with an stratified epithelium on top of a cellular stromal layer, and differed from the limbus in the absence of blood vessels and Vogt palisades. However, the presence of cytokeratin 15-positive cells in NANOULCOR, as it was the case of the limbus, suggests that NANOULCOR could partially resemble the phenotype of the scleral limbus, and partially retain a stemness profile. These resultscould contribute to explain why NANOULCOR has regenerative potential once grafted at the eye surface.

Acknowledgements: Supported by grants FIS PI23/00335 and ICI21/00010 (NANOULCOR), funded by Instituto de Salud Carlos III (ISCIII), Ministry of Science, Innovation and Universities (Plan Estatal de Investigación Científica, Técnica y de Innovación 2021-2023, and Plan deRecuperación, Transformación y Resiliencia), and co-funded by the European Union, Fondo Europeo de Desarrollo Regional ERDF-FEDER.

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P67 - Development of novel epithelial cell culture protocols using bioactive molecules with pro-proliferative potential

Ortiz-Arrabal O.[1,2], Campos A.[1,2], Etayo-Escanilla M.[1,2], Sánchez-Porras D.[1,2], GarcíaJ.M.[1,2], Martín-Piedra M.A.[1,2], Chato-Astrain J.[1,2], Carriel V.[1,2], Gómez-Llorente C.[1,2], Mesa-García M.D.[2,3,4], Alaminos M.[2,3,4]

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Introduction: Establishment of large amounts of cultured cells from human tissue biopsies is limitedby the low proliferation rate shown by certain cells, especially, human cornea and skin epithelial cells. For this reason, generation of bioengineered human cornea and skin substitutes by tissue engineering is a time-consuming process typically requiring 3-4 weeks. One of the possible methods contributing to establish epithelial cell cultures in a reduced time is the use of culture media enriched with bioactive molecules able to improve ex vivo cell proliferation [1]. In this regard,phenolic extracts obtained from virgin olive oil contain a mixture of bioactive molecules with a minimum of 40% of hydroxytyrosol, that previously demonstrated to have multiple health benefits [2]. In this study, we determined the potential effect of this phenolic extract for improving human epithelial cell culture protocolsin tissue engineering.

Methods: Human epithelial cells were cultured using aspecific culture medium enriched with different concentrations of phenolic extract (1-80 µg/mL) for 24, 48and 72 h. Cell viability was then evaluated using LIVE/DEAD and quantification of free DNA released to the medium, whereas cell proliferation was determined by WST-1 and cell counting at each analysis time using flow cytometry.

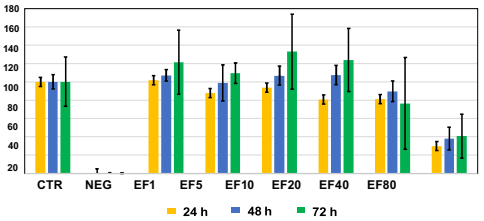

Figure 1. Cell proliferation as determined by WST1 activity of cultured epithelial cells using increasing concentrations of phenolic extract (EF) and controls.

Results: Cell viability analysis revealed that the highest concentrations of phenolic extract, specially40 and 80 µg/mL, was associated to a significant decrease of cell viability, as compared to controlcells, after 14, 21 and 28 days of culture, with a reduction in the percentage of live cells as determined by LIVE/DEAD and an increment of released DNA. Cell proliferation assays using WST1 activity and cell counting showed that the concentration of 10 µg/mL of phenolic extract wascapable of significantly increasing the proliferation rate of human epithelial cells after 72h of follow-up.

Discussion & conclusions: Our findings suggest that phenolic extract used at the concentration of 10 µg/mL can improve cell proliferation without impairing cell viability, and open the door to the useof this bioactive compound for the establishment of primary cultures of human epithelial cells extracted from corneal and skin biopsies for future use in tissue engineering protocols. Further research is needed to confirm the beneficial effects of these bioactive molecules in cornea and skintissue engineering.

Acknowledgements: Supported by grant PE-0395-2019 from Consejería de Salud y Consumo, Junta de Andalucía, Spain. Supported by grant FIS PI23/00335, funded by Instituto de Salud CarlosIII (ISCIII), Ministry of Science, Innovation and Universities (Plan Estatal 2021-2023, and Plan de Recuperación, Transformación y Resiliencia). Supported by grant C-CTS-032-UGR23, Plan Propiode Investigación y Transferencia de la Universidad de Granada 2023, Consejería de Universidad,Investigación e Innovación, Junta de Andalucía. Funded by the European Union, Fondo Europeo de Desarrollo Regional ERDF-FEDER, Programa Operativo FEDER Andalucía 2021-2027.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P68 - Histological assessment of a xenogeneic bone particle filler used in maxillofacial bone regeneration

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Introduction: Bone regeneration therapies applied to bone defects are of great interest in dentistryand maxillofacial surgery. The objective of this study was to evaluate the effectiveness of novel xenogeneic bone particle filler biomaterials using computed tomography and histological, histochemical and immunohistochemical analyses.
Methods: Male Wistar rats weighing 250 gr were used. In each animal, a critical bone defect of 5 mm in diameter was created at the mandibular angle. The experimental groups were: animals in which the bone defect was filled with a novel bone filler particles generated by deproteinizing bovinebone tissue (BOS-HA, TISSUM Biomaterials®, Córdoba, Argentina) (BH group); negative controls,in which the bone defect was allowed to heal without filler (CTR- group); and positive controls of native animals without any bone defect (CTR+ group). After 60 days, animals were euthanized, and the mandible defects were analyzed using computed tomography (CT). For the histological analysis, tissues were decalcified, embedded in paraffin and histologically analyzed.
Results: The CT scan analysis revealed that the size of the defect was smaller in BH (91.24 ± 35.90 %), as compared to CTR- (100 ± 35.06 %), but differences were not statistically significant ($p>0.05$). The histological analysis showed compact lamellar bone tissue in CTR+, while in CTR-, a loss bonetissue continuity was observed, where the defect had a thin band of connective tissue with the presence of collagen fibers, proteoglycans, and adipose tissue. In addition, regenerated bone tissue was observed around the grafted particles, and bone tissue regenerated was observed outside the limits of the mandibular bone. The presence of multinucleated giants in contact with thebone particles was also evidenced. The immunohistochemical analysis showed higher expressionof osteocalcin in BH than in CTR-.
Discussion & conclusions: The use of BH filler particles was able to increase the volume of connective and bone tissues at the bone defect site. Also, bone regeneration outside the limits of the mandibular bone compared to the control was observed [1]. These data correlated with the higher expression of osteocalcin and the CT scan images. While multinucleated giant cells were observed, there is debate over whether the deproteinized bone particles tended to degrade and become biointegrated at the host site [1-3]. These results suggest that xenogenic bone particles could partially contribute to bone regeneration in a more effective manner, and could be useful in the development of artificial tissues through tissue engineering techniques.
Acknowledgements: This work was supported by grants FIS PI21/00980 and ICI19/00024 (BIOCLEFT), funded by Instituto de Salud Carlos III (ISCIII), Ministry of Science, Innovation and Universities (Plan Estatal de Investigación Científica, Técnica y de Innovación 2021-2023, and Plande Recuperación, Transformación y Resiliencia), and co-funded by the European Union, Fondo Europeo de Desarrollo Regional ERDF-FEDER.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P69 - Generation of novel human cornea substitutes using fibrin-agarose hydrogels functionalized with cod collagen fibers

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Introduction: The human cornea is often afflicted by diseases and injuries that result in visual impairment. Current treatment options, such as corneal transplantation, are hindered by the lack ofdonors and immune rejection. Tissue engineering offers a promising solution, with notable advancements including the NANOULCOR anterior corneal substitute generated using fibrin- agarose hydrogels (FAH). Although these artificial corneas showed promising clinical results in patients with severe cornea damage [1], the biomaterial used in this model should still be improvedto obtain higher levels of biomimicry. The aim of this study is to generate functionalized FAH usingcollagen molecules isolated from natural industrial by-products (cod skin) to create novel cornea substitutes with increased biological properties.

Methods: Collagen was extracted from cod skin following previously reported protocols [2]. Briefly,skin was separated from the cod, washed, and subjected to pre-treatment with NaOH. Then it wassolubilized with acetic acid and pepsin treatment, precipitated with NaCl at pH 7.5 and lyophilized.Collagen was then solubilized in 0.1M acetic acid with 1 mg/ml of pepsin and it was mixed withFAH at different concentrations (2, 4, 8 and 10 mg/ml). Finally. human corneal epithelial cells and stromal keratocytes were included in these biomaterials and maintained during 1 week understandard cultured conditions. Histological evaluation was performed after 2 and 7 days of culture. Results: An optimal solubilization process for cod collagen was achieved, which enabled anefficient extraction and integration with FAH. Histological analysis showed a homogeneous stromacontaining corneal cells. Histological evaluation using hematoxylin and eosin showed somedifferences in epithelial cell distribution and keratocyte organization among the different corneal substitutes containing varying cod collagen concentrations. At day 2 of culture, epithelial cellsexhibited irregular distribution, forming a partial monolayer on top of the substitute. However,analysis of samples at day 7 of culture showed the formation of a well-developed epithelial layer, with signs of epithelial differentiation and maturation. Enhanced epithelial stratification wasparticularly notable, reaching optimal levels when cod collagen was used at a concentration of 8mg/ml in functionalized FAH.

Discussion & conclusions: These results show that functionalization of FAH with cod collagen is able to significantly improve the histological results and generate more biomimetic human cornea substitutes by tissue engineering. Promising effects were observed, including improved epithelial stratification and keratocyte organization. This approach offers a sustainable solution, aligned withenvironmental concerns, while addressing clinical needs. However, further optimization is necessary to achieve native-like tissue characteristics.

Acknowledgements: Supported by grants FIS PI23/00335 and ICI21/00010 (NANOULCOR), funded by Instituto de Salud Carlos III (ISCIII), Ministry of Science, Innovation and Universities (Plan Estatal de Investigación Científica, Técnica y de Innovación 2021-2023, and Plan deRecuperación, Transformación y Resiliencia), and co-funded by the European Union, Fondo Europeo de Desarrollo Regional ERDF-FEDER.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P70 - EDC/NHS crosslinking enhanced the physical properties of fibrin-agarose hydrogels for use in tissue engineering

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Introduction: Tissue engineering offers new opportunities for regenerative treatment for patients with damage of the skin, cornea, urethra and nerve. Previous tissue-engineered medicinal productsbased on fibrin-agarose biomaterials (FA) were developed, and promising preclinical and clinical results were achieved [1]. However, FA-based artificial tissues need further optimization and functionalization to obtain fully functional and biomimetic substitutes able to overcome the limitations of currently available tissues. Here, we evaluated novel chemical crosslinking agents onFA hydrogels in tissue engineering protocols of the human skin, cornea, urethra and nerve.

Methods: For FA crosslinking, a 10 mM solution of carbodiimide crosslinker (EDC) was combined with a 5 mM solution of N-hydroxysuccinimide (NHS) to produce three distinct final crosslinker solutions with ratios of [2:1], [3:1], and [4:1]. Subsequently, FA hydrogels underwent crosslinking for 6 hours followed by washing with water and PBS. The morpho-structural pattern of EDC/NHS-crosslinked FAH was analyzed using scanning electron microscopy (SEM), while biomechanical properties were assessed using an Instron analyzer to determine parameters such as Young's modulus, strain at fracture, and percentage of deformation. Furthermore, human dermal fibroblastswere seeded onto the surface of FA hydrogels for ex vivo analysis of biocompatibility using Cell Proliferation Reagent WST-1 and LIVE/DEAD (LD) cell viability/cytotoxicity analysis kit.

Results: SEM results revealed differences between the crosslinked scaffolds and the control group.When porosity was studied, differences were found between crosslinked hydrogels and control groups. Tensile testing findings demonstrated that crosslinking effectively modified the biomechanical properties of FA hydrogels, with differences among concentrations. Particularly, the[4:1] concentration significantly enhanced material resistance and load capacity. Finally, biocompatibility showed that human fibroblasts adhered to the crosslinked biomaterial without discernible differences compared to the FA group, as evidenced by the LD assay, which also indicated the absence of dead cells. Additionally, no significant differences were observed in the WST1 assays for the [3:1] and [4:1] crosslinking concentrations.

Discussion & conclusions: This study demonstrated the potential of EDC/NHS crosslinkers to enhance the biomechanical properties of FA hydrogels, especially with the [4:1] concentration. Theabsence of alterations in human cell adherence, cytotoxic changes, and metabolic activity supportsits non-toxic nature. These findings demonstrate the successful generation of scaffolds with enhanced physical properties tissue engineering applications. However, future studies should focuson developing a complete substitute of the human skin, cornea, urethra and nerve with histological properties resembling native structures showing in vivo functionality.

Acknowledgements: This work was supported by C-CTS-032-UGR23, Plan Propio de Investigación y Transferencia de la Universidad de Granada 2023, Consejería de Universidad, Investigación e Innovación, Junta de Andalucía. Funded by the European Union, Fondo Europeo de Desarrollo Regional ERDF-FEDER, Programa Operativo FEDER Andalucía 2021-2027, and grant PE-0395- 2019 from Consejería de Salud y Consumo, Junta de Andalucía, Spain. Supported by grants FIS PI23/00335, FIS PI23/00337, and FIS PI22/00059, funded by Instituto de Salud Carlos III (ISCIII),Ministry of Science, Innovation and Universities (Plan Estatal de Investigación Científica, Técnica y de Innovación 2021-2023, and Plan de Recuperación, Transformación y Resiliencia), and co- funded by the European Union, Fondo Europeo de Desarrollo Regional ERDF-FEDER.

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P71 - Histological evaluation of UGRSKIN tissue substitutes grafted in severely burnt patients

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Introduction: The surgical treatment of severely burnt patients is based on the use of autografts obtained from healthy areas of the patient's skin. However, these grafts are not always available, and side effects are very common at the donor site. The tissue engineering group previously developed and described a model of bioartificial human skin named UGRSKIN [1], which was grafted in patients affected by more than 60% of body surface severely burnt. In the present work,we have evaluated the UGRSKIN grafted in patients to determine the sequential process of maturation of the epithelial layer.

Methods: Skin biopsies were obtained from patients treated with the UGRSKIN model using local anesthesia, after 30, 60 and 90 days of the implant. Biopsies were fixed in formalin, processed forhistological analysis, and stained with hematoxylin and eosin. Then, samples were subjected to immunohistochemical analyses using specific antibodies for the epithelial markers cytokeratins 8 (CK8) and 10 (CK10) and involucrin (INV). Results were quantitatively analyzed using the softwareImageJ, and the intensity levels were compared with those obtained in normal, native skin, used as control (CTR).

Results: We first found that the epithelial layer of samplesgrafted in patients showed a well-differentiated epidermis from day 30 to day 90, with few differences with the follow-up time (Figure 1). From the beginning, the epithelium showed more than 20 cell strata, with detectable basal, spinosum, granulosum, and corneous layers, with evident signs of cell desquamation, comparable to CTR tissues. Immunohistochemical analysis of CK8 showed very few expression of this protein both in UGRSKIN and CTR samples, regardless the follow-up time. For CK10, the immunohistochemical staining signal was high in all samples, especially in CTR, although the expression levelswere significantly lower in UGRSKIN corresponding to 30 days of follow-up as compared to CTR, and reached similar levels to CTR at days 60 and 90. Finally, immunohistochemical analysis of INV revealed lower levels of expression than CK10, witha behavior that was very similar to this cytokeratin. Specifically, highest levels corresponded to CTR, with non-significant differences with samples at 60 and 90 days, and significant differences with samples at day 30 of development.

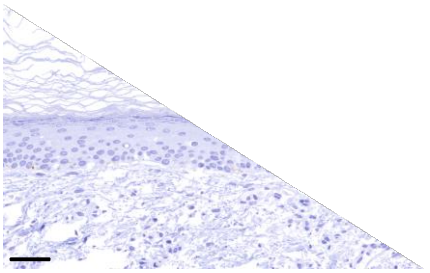

Figure 1. Histological analysis of UGRSKIN grafted in patients after 30 days of follow-up.

Discussion & conclusions: These findings suggest that UGRSKIN tissue substitutes are highly biocompatible and tended to integrate at the recipient site from day 30 of development. Theepithelial layer was devoid of any sign of histological alteration, supporting the biosafety of thetreatment. The rapid development of a protective epithelial layer able to express typical markerssuch as CK10 and INV could explain the positive clinical results found in severely burnt patients. Acknowledgements: This work was supported by grant PE-0395-2019 from Consejería de Salud yConsumo, Junta de Andalucía, Spain. Supported by grant C-CTS-032-UGR23, Plan Propio deInvestigación y Transferencia de la Universidad de Granada 2023, Consejería de Universidad,Investigación e Innovación, Junta de Andalucía. Funded by the European Union, Fondo Europeode Desarrollo Regional ERDF-FEDER, Programa Operativo FEDER Andalucía 2021-2027.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P72 - A novel human corneal substitute tailored with native extracellular matrix components for tissue engineering applications

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Introduction: Corneal diseases are among the main causes of blindness worldwide. Tissue engineering emerges as a new therapeutic strategy that aspires to improve current treatments. In this sense, an allogeneic tissue-engineered anterior lamellar nanostructured artificial human cornea named NANOULCOR was previously developed by our group using fibrin-agarose hydrogels (FAH) [1]. However, the composition and structure of this bioartificial tissue still needs further improvement in order to reproduce the fine histomolecular structure of the human native cornea. In the present study, we generated novel models of human bioartificial corneas improved by the use of native cornea decellularized extracellular matrix components (dECM) in order to create substitutes with heightened biological properties.

Materials and Methods: Porcine corneas were subjected to two decellularization protocols (P1 and P2). The P1 protocol was a reference protocol [2] and combined a chemoenzymatic-based solution,while the P2 protocol was a novel approach developed by the research group which reduced decellularization time in one day. To confirm proper decellularization, histological assessments, including hematoxylin and eosin (HE) and DAPI staining, along with quantification of DNA contentwere conducted to verify cellular removal and histochemical staining like Alcian Blue (AB) and Picrosirius red (PS) staining were performed to evaluate ECM preservation. Decellularized corneaswere then lyophilized and solubilized using 0.1M acetic acid and 1 mg/ml pepsin, followed by integration in FAH at the concentration of 2 and 4 mg/ml. Finally, human corneal epithelial cells and stromal keratocytes were cultured on the hydrogels, and histological evaluation was performed after 2 and 7 days.

Results: First, our initial histological analysis confirmed the effective removal of cellular elements and preservation of the overall corneal structure and essential ECM components using both decellularization protocols. Moreover, DNA content analysis demonstrated a significant reduction in decellularized corneas, meeting safety thresholds for potential clinical use in both protocols. P2was then selected for its ability to reduce decellularization time. Native corneal ECM demonstratedto be properly integrated with FAH, resulting in novel functionalized corneal substitutes. Histological examination of functionalized corneas revealed notable presence of keratocytes in the stroma andepithelial cell growth, with increasing stratification observed by day 7. Interestingly, the incorporation of corneal ECM led to enhanced epithelial stratification at the concentration of 4 mg/ml after 7 days.

Discussion & conclusions: Functionalization of FAH with solubilized native corneal dECM represents a significant advancement in the development of artificial corneal substitutes. These substitutes exhibited successful cell growth, with improvement in epithelial stratification. However, further refinement and optimization of this approach needs to be performed for the development of clinically relevant corneal substitutes with improved functional outcomes.

Acknowledgements: Supported by grants FIS PI23/00335 and ICI21/00010 (NANOULCOR), funded by Instituto de Salud Carlos III (ISCIII), Ministry of Science, Innovation and Universities (Plan Estatal de Investigación Científica, Técnica y de Innovación 2021-2023, and Plan de Recuperación, Transformación y Resiliencia), and co-funded by the European Union, Fondo Europeo de Desarrollo Regional ERDF-FEDER.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P73 - Evaluation of cryopreservation methods for long-term storage of bioengineered artificial oral mucosa in tissue banks

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Introduction: In the field of tissue engineering, preservation of artificial tissues is a crucial aspect that has garnered significant attention. Traditional preservation methods, such as refrigeration, arecommonly used in tissue banks to store human tissues at 4°C or deeply frozen for varying periods[1]. However, these methods can lead to significant alterations of extracellular matrix components, such as collagen fibrils and glycosaminoglycans, during cryogenic storage. Therefore, the development of effective cryoprotection agents and protocols is essential to ensure the integrity and functionality of artificial tissues.
Methods: 18 artificial oral mucosa stromas (AOMS) were prepared by tissue engineering, by seeding 1 x 10^5 cells in 1mL of fibrin-0.1% agarose hydrogels. AOMS were maintained in standardculture conditions with DMEM supplemented with 10% fetal bovine serum for 3 weeks. Then, 2 replicates of AOMS were cryopreserved in 3 different cryoprotectants (10% DMSO, 0.3 M trehaloseand Cryostor©) for 30 days, at 4ºC, -20ºC and -80ºC of temperature. Tissues were then thawed, conditioned in DMEM at 37ºC for 24 hours and analyzed to determine cell viability by WST-1 assays. Structural stability was assessed by hematoxylin-eosin histological analysis.
Results: In terms of cell viability none of the evaluated experimental groups was able to effectivelycryopreserve cells inside the AOMS at 4ºC (refrigeration temperature), as WST-1 activity was verylow. However, when AOMS were freezed at -20ºC and -80ºC, viability was maintained in the DMSOand Cryostor© groups, whereas trehalose cryopreservation resulted in a loss of cell viability, independently of the temperature. Specifically, at -20ºC, Cryostor© preservation leaded to more viable AOMS as compared to the DMSO group. Freezing at -80ºC with DMSO and Cryostor© also resulted in viable cells, with higher values found for DMSO. Structural stability showedthat AOMS cryopreserved in DMSO and Cryostor© had adequate cell density and integrity of the hydrogel, especially in the Cryostor© group. AOMS cryopreserved with trehalose at -20ºC and -80ºC showed a decreased cell density as well as some fragmentation of the scaffold.

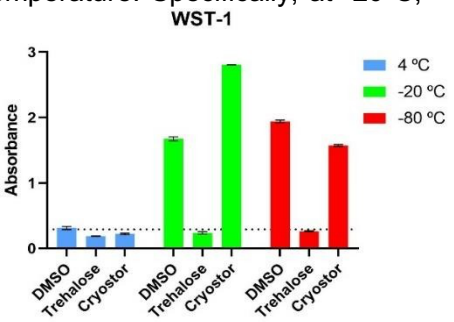

Figure 1. Cell viability in AOMS assessed by WST-1 assay (blank value is represent as dotted line)

Discussion & conclusions: Our results suggest that cryopreservation in DMSO showed the best preservation in terms of cell viability and structural stability of AOMS, especially when bioengineered tissues are stored frozen, not refrigerated. Also, the commercial cryoprotectant agent Cryostor© achieved good stability after 30 days of freezing. Although these results are preliminary and should be confirmed in a larger cohort of bioengineered tissues, the present work supports the possibility of cryopreserving human bioartificial tissues in tissue banks for delayed use in patients with severe alterations of the oral cavity.
Acknowledgements: This work was supported by grants ICI19/00024 (BIOCLEFT) and FIS PI21/00980, funded by Instituto de Salud Carlos III (ISCIII), Ministry of Science, Innovation and Universities (Plan Estatal de Investigación Científica, Técnica y de Innovación 2021-2023, and Plande Recuperación, Transformación y Resiliencia), and co-funded by the European Union, Fondo Europeo de Desarrollo Regional ERDF-FEDER.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P74 - Novel Biogenic Conduits for Use in Tissue Engineering: Generation, Decellularization, and *Ex Vivo* Characterization

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Introduction: Despite the availability of diverse conduits, there is still a need to generate tubular substitutes to treat peripheral nerve, blood vessel or urethral injuries. This is largely due to the intrinsic structural complexity and molecular composition of these extracellular matrices, being a challenge to replicate similar structures synthetically. Consequently, researchers are driven to explore alternative biofabrication techniques to generate tissues or matrices with greater biomimicry for a wide range of tissue engineering applications. The present study is aimed to create*in vivo* bio-artificial conduits by stimulating a encapsulation response through the implantation of anon-resorbable material in experimental animals. This procedure generates a tubular substitute with histological complexity beyond what is achievable through current artificial biofabrication methods. The objective was to enhance the immunological and biomechanical properties of thesebiogenic conduits through decellularization and chemical cross-linking, respectively.
Methods: A silicone tube was surgically inserted into the dorsal region of 25 Wistar rats to facilitatethe formation of a connective tissue sheath around implanted tube, referred to as the bioconduit. After 4 and 8 weeks post-implantation, the bioconduits were harvested and subsequently decellularized by using a chemo-enzymatic method previously employed in peripheral nerves [1]. To enhance the biomechanical properties of the bioconduits, crosslinking with the natural agent genipin (GP) was performed. The native, decellularized, and GP-crosslinked bioconduits were subjected to histological, *ex vivo* biocompatibility, and biomechanical analyses.
Results: Histology (using HE technique) showed consistent structural uniformity in the bioconduitsafter 4 and 8 weeks post-implantation, maintaining their overall structural integrity following decellularization and genipin crosslinking treatments. It was observed an increased of the thicknessof the time, especially within the inner surface and an efficient decellularization (HE and DAPI) in all groups. Picrosirius staining showed no remarkable changes in the collagen content and patternbetween groups. Interestingly, the GP crosslinking significantly increased the resistance values ofdecellularized bioconduits under tensile test. Moreover, *ex vivo* biocompatibility assessment, by using human fibroblast and Live/Dead assay, showed positive results in all groups even those treated with genipin.
Discussion: This study demonstrates the possibility to generate highly complex acellular tubular matrices by implanting non-biodegradable molds within the connective tissue of laboratory animal models and decellularization techniques. Better structural and biomechanical properties can be obtained with the use of GP as crosslinking agent. However, in vivo studies are still needed to determine the repair or regeneration capabilities of these matrices in neural, vascular or urethral tissue engineering applications.
Fundings: Supported by grants FIS PI23/00337 and FIS PI22/00059, funded by Instituto de SaludCarlos III (ISCIII), Ministry of Science, Innovation and Universities (Plan Estatal 2021-2023, and Plan de Recuperación, Transformación y Resiliencia). Co-funded by the European Union, Fondo Europeo de Desarrollo Regional ERDF-FEDER. Funded by grant CPP2021-009070 by “Proyectosde colaboración público-privada”, Plan de Investigación Científica, Técnica y de innovación 2021-2023, Ministerio de Ciencia e Innovación, Unión Europea, Agencia Estatal de Investigación, España.
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Histology and Histopathology
From Cell Biology to Tissue Engineering

P75 - Assessing fibroblasts role in neural cancer invasion using a 3D model developed by tissue engineering

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Introduction: Invasion and metastasis are critical aspects of cancer progression, significantly contributing to disease morbidity and mortality. While lymphatic and hematogenous metastatic pathways received much research attention, neural invasion (NI) remains a relatively understudiedand poorly comprehended aspect of tumor dissemination, despite its clinical relevance. In this sense, our group recently developed an in vitro NI model by tissue engineering, by combining the complexity of a nerve segment with cancer cells in a biomimetic fibrin-agarose hydrogel system [1].Now, we used this model to incorporate human skin fibroblast cells to study their influence on NI progression.
Methods: Human epidermoid carcinoma cells (A431), human skin fibroblasts, and rat sciatic nerves were used for the construction of the NI models. Two distinct variants of NI models were developed,following published protocols [1]. One variant included normal human fibroblasts within the stromaco-cultured with cancer cells and a nerve segment (cInv), while the other group was devoid of human fibroblast cells (Inv). These models were cultured for 2 and 7 days using standard culture conditions. Subsequently, the NI models underwent histological, histochemical, and immunohistochemical evaluation to characterize the distribution and phenotype of cells within the neural microenvironment. Alcian blue (AB) and Picrosirius red (PS) histochemical techniques were employed to analyze extracellular matrix composition, while immunohistochemical staining for cytokeratin 5 (CK5), an epithelial cell marker, was conducted to determine cell profiling.
Results: First, our study demonstrated that the combination of cancer cells with a nerve segment in a fibrin-agarose hydrogel successfully recreated NI features in vitro, recapitulating key aspects of cancer cell aggregation and proliferation. Importantly, the nerve stroma was maintained withoutsignificant changes, which allowed us to study the interaction between cancer cells and the complex neural microenvironment. Furthermore, histological analysis revealed alterations in cancerproliferation dynamics and cellular distribution patterns. Specifically, the inclusion of normal humanfibroblast cells influenced cancer aggregate formation and diffusion towards the nerve segment, suggesting a modulatory role in the invasive process.
Conclusions: This study demonstrates the feasibility of generating a sophisticated in vitro model of NI using a biomimetic hydrogel system. The preservation of nerve stroma integrity makes thismethod a valuable tool for studying neural invasion progression. Additionally, our findingshighlighted the influence of the inclusion of human normal fibroblast cells on cancer cell behavior within the neural microenvironment, warranting further investigation into their role in NI dynamics.
Acknowledgments: Supported by grant PPJIA2022-19, Plan propio de investigación y transferencia 2022, Universidad de Granada, España. Supported by grant FIS PI23/00337, funded by Instituto de Salud Carlos III (ISCIII), Ministry of Science, Innovation and Universities (Plan Estatal de Investigación Científica, Técnica y de Innovación 2021-2023, and Plan de Recuperación,Transformación y Resiliencia), and co-funded by the European Union, Fondo Europeo deDesarrollo Regional ERDF-FEDER. Grant CPP2021-009070 by “Proyectos de colaboración público-privada”, Plan de Investigación Científica, Técnica y de innovación 2021-2023, Ministeriode Ciencia e Innovación, Unión Europea, Agencia Estatal de Investigación, España.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P76 - Functionalizing Biomaterials for Enhanced Bone Regeneration using Holothurian Ossicles

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Introduction: Recent advancements in dental tissue engineering and biomaterials have led to the development of new strategies for soft and hard tissue repair. However, treating hard tissues like bone presents significant challenges due to their unique properties. Traditional treatments for severe bone injuries involve human bone grafts, lyophilized animal bone, or hydroxyapatiteparticles, but their integration into bone is slow, resulting in low success rates. Therefore, there is a pressing need for developing highly biocompatible materials to repair bone defects. Holothurians,a type of echinoderms, possess calcified ossicles in their skin, which offer potential as biomaterialsdue to their unique microstructures providing both flexibility and strength [1]. In this work, we haveisolated holothurian ossicles in order to functionalize fibrin-agarose biomaterials for bone regeneration applications.

Methods: Holothurian endodermal ossicles particles were obtained by treating the skin of specimens of *Holothuria sp.*using 5% hypochlorite for 4-6h to disaggregate the organic tissues and release the inorganic particles to the solution. Holothurian ossicles were harvested and rinsed in distilled water to remove all rests of sodium hypochlorite. Ossicles were washed in 70% ethanol for sterilization, and dried. Subsequently, Holothurian ossicles were co-cultured with $2x10^5$ human oral mucosa fibroblasts in a bidimensional system and in a tridimensional system using 0.1% fibrin withspongecol® biomaterials. All groups were maintained under *ex vivo* culture conditions for 7 days in order to histologically evaluate ossicles-matrix and ossicles-cell interactions.

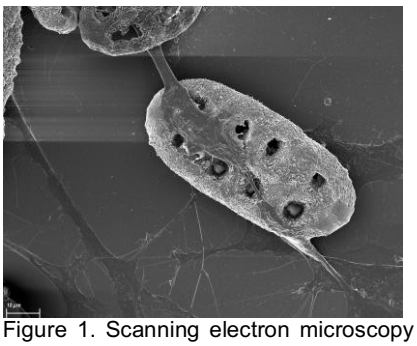

Figure 1. Scanning electron microscopy imagen of bidimensional co-culture of human oral mucosa fibroblasts with holothurian ossicles.

Results: Our results showed that cells co-cultured with holothurian ossicles were viable, with no morphological or functional alterations derived from the presence of the ossicles in the two-dimensional and three-dimensional systems, suggesting that these biomaterials could be highly biocompatible (Figure 1). Likewise, our results from optical andelectron microscopy demonstrated that the cells maintained their viability within the artificial tissues,with no morphological or functional alterations detected. It is important to highlight that the particleswere mainly concentrated at the base of the 0.1% fibrin and spongecol® biomaterials, probably dueto their tendency to settle in liquid media.

Discussion & conclusions: The use of natural biomaterials derived from holothurians has proven tobe highly biocompatible and likely to yield better results in terms of bone regeneration than other materials currently available. Its natural origin, sourced from highly abundant marine life, providesa potential benefit as an enhancer of fishing communities' development. Furthermore, in the near future its potential clinical use could be focused on treating patients with severe bone injuries requiring the implantation of bone regeneration-enhancing materials.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P77 - Optimization of mesenchymal stem cells cultures using xenogeneic-free conditions

Garzón I.[1,2], Pérez G.[1], Martín-Piedra M.A.[1,2], Chato-Astrain J.[1,2], España-López A.[3,4], Ortiz-Arrabal O.[1,2], Fernández-Valadés R.[1,2,3,5], Alaminos M.[1,2], Sánchez-Quevedo M.C.[1,2]

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Introduction: Mesenchymal Stem Cells (MSCs) are increasingly used in tissue engineering, requiring in vitro expansion fulfilling good manufacturing practice (GMP) guidelines. Although mostcell culture protocols rely on fetal bovine serum (FBS), this component should be replaced, due tohigh variability, contamination risk, and immunization concerns. In fact, the European Agency for the Evaluation of Medicinal Products recommends replacement of FBS with alternative culture methods. In this context, current research is focused on human blood-derived components including human plasma, serum, umbilical cord blood serum, and platelet derivatives, as culture media supplements [1]. In this work, we have performed an optimization of the use of human plasmaas a supplement in cell cultures of dental pulp and Wharton jelly MSCs, in order to enhance cell culture conditions in GMP facilities.
Methods: Human MSCs derived from human dental pulp (DPSCs) and umbilical cord (WJSCs) were obtained and expanded according to stablished protocols. Once cells reached 70% of confluence, cells were detached into a single-cell suspension using 0,25% trypsin and subculturedin cell culture chambers at a cell density of $1x10^4$ cells/ml during 72 hours in DMEM and 1% antibiotics and supplemented with fetal bovine serum (FBS) and human plasma (HP) at different concentrations (0%, 1%, 5% and 10%). Histomorphological and cell viability analyses were then performed to assess the effect of each supplement.
Results: Both supplements (FBS and HP) supported MSC growth after 72 hours, and cell cultureswere established and maintained with no detectable differences between both groups of study. When cell viability was analyzed, we found that FBS and HP cultures were associated to high cellviability indexes, with no differences between both groups, supporting that both products were highly biocompatible for *ex vivo* use. However, differences in cell morphology were evident in WJSCs cultured with HP supplemented media at all concentrations (1%, 5% and 10%), with cells showing a spindle-shape, elongated morphology.
Discussion & conclusions: Our preliminary study demonstrated that the use of HP as media supplement enables MSCs expansion without compromising cell viability and proliferation. The useof this medium could contribute to prevent the drawbacks of the use of FBS, including humoral response after repeated administrations at in vivo levels, or the possibility of transmitting diseasesfrom the animal to the host patient. Future studies should be carried out using alternative cell sources to determine the potential of HP as culture supplement.
Acknowledgements: This work was supported by C-CTS-032-UGR23, Plan Propio de Investigación y Transferencia de la Universidad de Granada 2023, Consejería de Universidad, Investigación e Innovación, Junta de Andalucía. Funded by the European Union, Fondo Europeo de Desarrollo Regional ERDF-FEDER, Programa Operativo FEDER Andalucía 2021-2027, and grant PE-0395- 2019 from Consejería de Salud y Consumo, Junta de Andalucía, Spain. Supported by grants FIS PI21/00980, FIS PI22/00059 and FIS PI23/00335, funded by Instituto de Salud Carlos III (ISCIII), Ministry of Science, Innovation and Universities (Plan Estatal de Investigación Científica, Técnica y de Innovación 2021-2023, and Plan de Recuperación, Transformación y Resiliencia), and co- funded by the European Union, Fondo Europeo de Desarrollo Regional ERDF-FEDER. [1] Griffiths S, Baraniak PR, Copland IB, Nerem RM, McDevitt TC. Human platelet lysate stimulates high- passage and senescent human multipotent mesenchymal stromal cell growth and rejuvenation in vitro. Cytotherapy. 2013 Dec;15(12):1469-83.

Histology and Histopathology
From Cell Biology to Tissue Engineering

P78 - Fabrication of a preliminary substitute of the human urethra corpus spongiosum by tissue engineering

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Introduction: The corpus spongiosum is one of the erectile layers surrounding the human spongy urethra. Consisting of connective tissue containing numerous blood vessels, its main function is toprevent urethral collapse during erection. This structure is susceptible to traumatic, infectious, neoplasia and congenital conditions [1]. Clinical management of these cases is challenging, especially due to the lack of available tissue for grafting and the high risk of post-surgical stenosis and fistulation. For these reasons, generation of biological substitutes of this tissue could significantly contribute to the treatment of patients with severe damage at this level. In this regard,Tissue Engineering appears as a promising alternative for the fabrication of functional tissue available for clinical use. In the present work we have generated a preliminary model of corpus spongiosum tissue by combining specific cells of this layer and biocompatible biomaterials.
Methods: To generate a preliminary model of corpus spongiosum tissue, human microvascular cells were combined with fibrin hydrogels. In short, human plasma was mixed with cultured cells, tranexamic acid (to prevent fibrinolysis), DMEM culture medium and calcium chloride (to induce hydrogel polymerization), aliquoted in culture dishes and allowed to jellify in a cell incubator at 37ºC. After 2, 7 and 14 days of ex vivo culture, the viability of the cells immersed in the biomaterialof these corpus spongiosum substitutes was assessed using Live/Dead assays, and the pattern ofcell distribution and expression of vessel-specific markers was histologically evaluated using hematoxylin-eosin staining and CD31 immunohistochemistry.
Results: Cell viability analyses using Live/Dead showed that cells immersed within the fibrin hydrogels retained high percentages of viability, with more than 90% cell viability found at each study time (2, 7 and 14 days). Histological analysis revealed a homogeneous cell distribution, withcells scattered and spread along all the biomaterial. Interestingly, cells tended to self-organize, forming circular capillary-like patterns that showed positive immunohistochemical reaction for CD31, especially, after 7 days of development.
Discussion & conclusions: Our preliminary results suggest that this complex tissue can be reproduced in the laboratory using tissue engineering methods and protocols. This corpus spongiosum tissue substitute based on fibrin hydrogel biomaterials was able to promote vascular cell organization, and suggest that cells could be phenotypically functional, as determined by the positive expression of CD31 vascular markers. Future studies should determine the biocompatibility potential of these tissue substitutes generated by tissue engineering.
Acknowledgements: This work was supported by grant FIS PI22/00059, funded by Instituto de Salud Carlos III (ISCIII), Ministry of Science, Innovation and Universities (Plan Estatal de Investigación Científica, Técnica y de Innovación 2021-2023, and Plan de Recuperación, Transformación y Resiliencia), and co-funded by the European Union, Fondo Europeo de Desarrollo Regional ERDF-FEDER.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P79 - Generation of a fibrin-agarose substitute of the tunica albuginea of the human urethra

Sánchez-Porras D.[1,2], Campos F.[1,2], Chato-Astrain J.[1,2], Oyonarte S.[1,2,3], Liceras- Liceras E.[4], Díaz-Moreno E.[4], Bermejo-Casares F.[1], Fernández-Valadés R.[1,2,4], Sánchez-Quevedo M.C.[1,2]

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Introduction: The tunica albuginea is a dense, fibrous layer that surrounds both the urethra and thecorpora cavernosa of the penis and testicles. With leiomyocytes in its composition, the tunica albuginea participates in the contraction process that allows micturition and ejaculation. Thus, traumatic lesions or infections in this layer may lead to urethral stricture, which can cause pain, inability to urinate or anejaculation [1]. Nowadays, mucosal grafting is the gold standard treatmentin cases of urethral stricture. In the present work, we have generated an artificial tunica albugineasubstitute by combining leiomyocytes and fibrin-agarose scaffolds that previously showed excellentresults when applied to other tissues [2].
Methods: An artificial tunica albuginea was generated by encapsulation of cultured human leiomyocytes in fibrin-agarose hydrogels, and these constructs were maintained during 14 days ina cell incubator. Samples were then collected, processed and paraffin embedded for histochemicalanalysis by hematoxylin-eosin and immunohistochemical detection of basal lamina collagen type IV and the smooth muscle specific marker smooth-muscle-actin (SMA).
Results: Histological analysis using hematoxylin-eosin staining showed the presence of large cellsshowing positive SMA expression, which were homogeneously distributed throughout the fibrin- agarose hydrogels. Interestingly, the cells changed from a rounded shape at the first evaluation time (day 2) to a more elongated shape on subsequent days (7 and 14 days), thus mimicking the elongated shape of native leiomyocytes in human tissues.
Discussion & conclusions: In this work, we successfully generated a preliminary substitute of the human tunica albuginea by encapsulating leiomyocytes in fibrin-agarose hydrogels. These bioartificial tissues were able to mimic some of the features of the native tissue, and resembled thehistomorphological characteristics of smooth muscle cells. However, further analyses are needed to determine, *in vitro* and *in vivo*, the functional capabilities of these constructs.
Acknowledgements: This work was supported by grant FIS PI22/00059, funded by Instituto de Salud Carlos III (ISCIII), Ministry of Science, Innovation and Universities (Plan Estatal de Investigación Científica, Técnica y de Innovación 2021-2023, and Plan de Recuperación, Transformación y Resiliencia), and co-funded by the European Union, Fondo Europeo de Desarrollo Regional ERDF-FEDER.

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P80 - Generation and characterization of novel human urethral mucosa substitutes by tissue engineering

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Introduction: The urethra is a duct responsible for the transport of urine from the bladder to the exterior. Due to its function and location, it presents several peculiarities, such as the presence of a mucous tunica characterized by the existence of a specialized epithelium that can be transitional(urothelium) or stratified. The urothelium is a specialized variety of epithelium responsible for preventing the diffusion of toxic substances present in the urine into the stroma, while being able to modify its cellular morphology during the micturition process [1]. These differential characteristicsfrom other epithelia make it a more difficult variety to culture and maintain *ex vivo* for tissue engineering purposes. Although preliminary, previous studies from the research groupdemonstrated that bioartificial mucosa substitutes can be generated in the laboratory using natural biomaterials, with promising results [2].
Methods: To generate a substitute of the urethral mucosa, human fibroblasts were first combined with fibrin hydrogels to build a bioartificial stroma and then, human urothelial cells were seeded ontop to form an epithelial layer. After 2, 7 and 14 days of development, the viability of this mucosa substitute was determined by using Live/Dead tests, while histochemical methods were performedto determine the presence and distribution of fibroblast in the stroma, as well as the synthesis of urothelial cells and basement membrane components.
Results: Viability results demonstrate the presence of viable cells during the 14 days of in vitro culture. Histological results reveal a homogeneous fibroblast distribution in the bioartificial stroma,and the formation of a multiple layer of epithelial cells on top of this stroma. These analyses revealed that these mucosa substitutes were able to synthesize collagen IV and laminin, main components of basement membrane.
Discussion & conclusions: These results demonstrate the feasibility of generating a urethral tunicamucosa by tissue engineering protocols. This tissue substitute showed the main histoarchitecturalpatterns of native tissues, along with basement membrane components. However, future experiments are needed to determine the expression of specific urothelial markers, as well as the in vivo behavior of these artificial tissues. In addition, the putative clinical usefulness of bioartificialurethral mucosa generated by tissue engineering should be determined in patients with urethral lesions.
Acknowledgements: This work was supported by grant FIS PI22/00059, funded by Instituto de Salud Carlos III (ISCIII), Ministry of Science, Innovation and Universities (Plan Estatal de Investigación Científica, Técnica y de Innovación 2021-2023, and Plan de Recuperación, Transformación y Resiliencia), and co-funded by the European Union, Fondo Europeo de Desarrollo Regional ERDF-FEDER.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P81 - Tissue response of antibacterial polypropylene meshes for hernia repair tailored with rifampicin-loaded electrospun microfibers.

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Introduction: Tailoring biomaterials with antibacterial coatings represent a striking, constantly evolving strategy aimed at reducing/preventing risk of postoperative infections. In hernia repair, combination of antimicrobials and drug release systems have led to the development of cutting- edge prophylactic coatings for synthetic meshes with potential application. In this study, we have preclinically evaluated a novel antibacterial coating for synthetic biomaterials commonly used in surgical hernia repair.
Methods: Microfibers made of a carboxymethylcellulose/polyvinylalcohol mixture (CMC/PVA) and rifampicin (RIF) were electrospun on both sides of a lightweight polypropylene (PP) mesh for herniarepair, establishing 3 study groups: control (uncoated), ES (drug-free coating), and ES+RIF (antibacterial coating). Drug release (HPLC), antibacterial activity (*Staphylococcus aureus*; sequential agar diffusion test, sonication) and cytotoxicity (rabbit fibroblasts; alamarBlue) were tested *in vitro* (1cm^2 fragments). Then, performance of control, ES and ES+RIF meshes (5x2cm; n=6 each) was preclinically evaluated using a rabbit model of hernia repair inoculated under settingof infection (*S. aureus;* 10^6 CFU). At 14 postoperative days, response of implants in terms of antibacterial effect and biocompatibility (macroscopic outcomes, sonication, light/scanning electronmicroscopy, RAM-11 immunolabeling for macrophages) were assessed.
Results: *In vitro*, electrospun microfibers yielded a peak release of RIF after 8 hours, followed by ashort, sustained release for around 48 hours. While both control and ES meshes did not display any activity, antibacterial devices fully avoided adhesion to surface and developed inhibition halos(31.50 ± 0.561 mm) whose amplitude vanished by day 7. Any toxic effect to cultured fibroblasts was recorded. *In vivo*, performance of ES+RIF was demonstrated both macro and microscopically.By day 14, surface of implants was free of bacteria (100% clearance) being these meshes fully integrated into a loose neoformed tissue. Contrary, high loads of bacteria were recorded in both control (average: 8.14 x 10^5 CFU/cm^2) and ES implants (average: 3.4 x 10^5 CFU/cm^2) together withan impaired host tissue integration. Macrophage response was similar in all groups.
Discussion & conclusions: Providing PP meshes for hernia repair with drug-releasing coatings is a straightforward strategy aimed at reducing risk of postoperative infections. In our study, the coating manufactured by electrospinning successfully prevented bacterial adhesion, precluding earlydevelopment of implant infection. Likewise, structure of microfibers did not alter host tissue integration and compound composition was safe to cells and tissues, bringing to light the potentialusefulness of this novel coating to prevent bacterial colonization of implants.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P82 - Assessing new collagen therapies for wound healing: a murinemodel approach

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Introduction: The wound repair process is traditionally classified into four sequential but overlappingstages: haemostasis, inflammation, proliferation, and remodelling. Collagen is the major protein ofthe extracellular matrix of connective tissue in mammals and plays an important role in wound healing. In this study, we evaluated the efficacy of new collagen-based products not yet on the market (both hydrolysed and nonhydrolysed) and compared them to commercial products in a murine model of cutaneous healing.
Methods: Circular excisional defects with 1.5 cm of diameter were generated on the dorsal skin ofWistar rats (n = 72). Animals were randomnly distributed into five study groups (n = 12 each) according to the administered treatment, while the sixth group was a nontreated control group (*Table*).Treatments were systematically applied at predetermined time (0, 3, 5, 7, and 9 days). Six animals in each group were humanely euthanized in a CO_2 inhalation chamber 7- and 18- days post-surgery. The evolution of wound closure was macroscopicallyassessed by cenital photographs of the defects and scar tissue formation, at different time-points (immediately post-surgery, time of collagen application, and designated endpoint days). These measurements were used to calculate the relative values of the epithelialization progress, contraction, and wound closure. For morphological and histological analyses, tissue samples were stained with hematoxylin–eosin, Masson's trichrome, and Sirius red.The data were expressed as the mean ± standard deviation.
Results: After 7 days, open areas and the degree of epithelialization were similar among the groups, with no significant differences between them. However, significant differences were observed in contraction between the control group and groups treated with non-hydrolyzed collagen, both newly synthesized and yet marketed (Helix3-CP®) (*p<0.01* and *p<0.05*, respectively). In contrast, epithelialization process was similar in all groups.

Study groups	Amount (g)	Treatments
Control	-	No treatment
T1	0.05	New collagen-based semi-denatured (new)
T2	0.06	Catrix® non-hydrolysate (commercial)
T3	0.06	New collagen-based hydrolysate powder (new)
T4	0.05	New collagen-based non-hydrolysate (new)
T5	0.03	Helix3-CP® non-hydrolysate (commercial)

Table. Description of the different study groups and treatments

Both hematoxylin-eosin and Masson's trichrome staining revealed that untreated animals exhibited more pronounced development of vascularized and cellular granulation tissue, with a high numberof inflammatory cells and a disorganized extracellular matrix, along with type III collagen deposition(revealed by the yellow color of the Sirius Red stain). After 18 days, animals treated with new collagen (both native and non-hydrolyzed) exhibited accelerated wound closure, increased epithelialization and more organized granulation tissue.
Discussion & conclusions: Our morphometric and histological results suggest that local administration of new collagen (both hydrolysed and native) promotes the progression of the reparative process and significantly accelerates wound closure compared with nontreated wounds.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P83 - Usefulness of a tissue-engineered palate mucosa to prevent maxillofacial growth alterations in laboratory animals

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Introduction: Cleft palate is a common congenital malformation affecting the maxillofacial structure. Currently, the gold-standard treatment is based on the use of autografts obtained by bone denudation that are placed at the site of the palatal defect in order to create a separation between the oral and nasal cavities [1]. However, this treatment is typically associated to growth and development alterations of the maxillofacial bones, due to the denudations generated by the surgical procedure. In the present work, we describe a novel substitute of the palate mucosa generated by tissue engineering that demonstrated potential usefulness in laboratory animals. Methods: A tissue-engineered palate mucosa substitute called BIOCLEFT was developed using two types of cells isolated from a small biopsy of the palate mucosa (keratinocytes and fibroblasts). These cells were combined with nanostructured fibrin-agarose biomaterials previously designed by the research group. Fibrin was obtained from healthy donors of plasma, and commercially available type VII agarose was used at a final concentration of 0.1%. This tissue substitute was cultured for 3-4 weeks. BIOCLEFT substitutes were grafted in young laboratory rabbits in which a defect had been created at one side of the palate, resembling the denudation generated during the surgical repair typically applied to cleft palate children. Results were then evaluated after 6 months of follow-up using CT scanning, and morphometric analysis. Results: Evaluation of the structure of the BIOCLEFT product confirmed the presence of an epithelium on top of an artificial lamina propria consisting of abundant fibroblasts immersed in the fibrin-agarose biomaterial (Figure 1). Histochemical and immunohistochemical analyses confirmed the expression of human cytokeratins 4 and 13 both ex vivo and in vivo. At the stromal level, BIOCLEFT showed high contents of collagen fibers and proteoglycans, after in vivo grafting in laboratory rabbits, although at lower levels than native palate mucosa. Morphometric analysis revealed that control animals in which an artificial tissue was not grafted showed several maxillofacial bone growth alterations, with a significant dissymmetry between both sides of the palate bone ($p<0.05$). However, the use of BIOCLEFT could prevent these alterations, and palate bone development was more harmonic and symmetric. Discussion & conclusions: Novel regenerative therapies based on the use of tissues and organs generated by tissue engineering are contributing to the treatment of complex conditions, such as cleft palate. In the present work, we demonstrated the potential of a tissue-engineered palate mucosa to prevent the growth alterations found in animals with a palate defect. These preclinical results support the clinical application of this technology in patients with cleft palate.

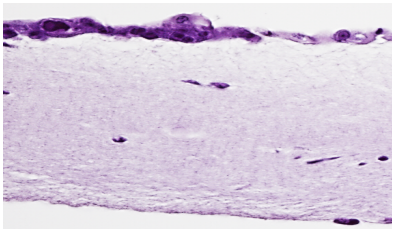

Figure 1. Histological analysis of the palate mucosa generated by tissue engineering using fibrin-agarose biomaterials.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P84 - Use of digital tools in the histopathology laboratoryas a means to reinforce the acquisition of practical skills in university students

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The acquisition of practical skills is one of the main problems faced by undergraduate university students. This difficulty is aggravated when we focus on subjects such as histopathology in healthsciences degrees, where an interconnection must be made between the contents of other subjectswith what is acquired in this one. For this reason, our group of teachers has implemented the use of free platforms that are easily accessible to students, such as the learning games platform. This platform allows the student, through a mobile phone device, to access the activities proposed during the practical sessions. In this sense, our group of teachers has assessed how in the last twoyears (2022-2023 and 2023-2024) this tool can be useful. We have a total of 40 students per practical session. In the 2022-2023 academic year, we carry out a group of practices without the use of digital platforms (NPD), as opposed to another where we implement the use of digital platforms (PD). In the 2023-2024 academic year, a new modification was made, where students are grouped into subgroups, and we carried out small competitions during the sessions through theuse of the digital platform (SPD). The analysis of the acquisition of skills was reflected in an increasein overall scores and satisfaction levels in the practical sessions in the SPD group. All of this allowsus to attest to the need to dynamically carry out practical sessions to improve the acquisition of skills and improve the integration of the multidisciplinarity of histopathology.

Keywords: Histopathology, digital platform, academic and practical.

Histology and Histopathology
From Cell Biology to Tissue Engineering

P85 - Evaluation of a master program in in Manufacturing of Advanced Therapy Medicinal Products

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Introduction: With the onset of tissue engineering and advanced therapies, histology has significantly widened its goals and objectives, and current histologists are now able to generate human tissues and organs as advanced therapied medicinal products (ATMP). However, very few formative programs in tissue engineering have been implemented in Europe, especially regardingprofessional qualification to work in GMP facilities. In this milieu, the University of Granada and theAndalusian Network for the design and translation of Advanced Therapies (And&tAT) of Junta de Andalucía launched in 2009 a training program in manufacturing of advanced therapy medicinal products [1]. This program is taught in English language since 2012, thus allowing international students to enroll and qualify. The University-specific Degrees offered are a Master Degree in Manufacturing of Advanced Therapy Medicinal Products (1,500 hours) and a Specialization Degreein Manufacturing of Advanced Therapy Medicinal Products (970 hours). Teaching includes online theoretical training with continuous support from instructors, and practical training in a GMP facilitydesigned for the manufacture of advanced therapy medicinal products [2].
Methods: Results obtained by students enrolled in the 7th edition of this formative program were evaluated using a normalized questionnaire elaborated by the University of Granada. This questionnaire included several questions that the students had to rate using a Lickert-like scale ranging from 1 (very low) to 5 (very high). Questions were related to the online teaching platform, teaching resources, tuition fees, organization of the courses, publicity of the program, duration of contents, communication with the teachers, fulfilling the objectives, contents, teachers, evaluationmethods and practical teaching. Students were also inquired about the specific aspects of the teaching program that they would modify.
Results: Average results were always above 4 points, with the following average scores obtained for each group of questions: platform (4.92), resources (4.83), fees (4.33), organization (4.75),
publicity (4.33), duration (4.83), communication (4.92), objectives (4.92), contents (4.92), teachers(5.00), evaluation (4.83) and practical teaching (4.83). One third of the students declared that theywould not change any aspect of the program, whereas two thirds suggested some improvement measures. These measures are related to an increase of the practical contents (25% of the students), improvement of the schedule (16.67%), improvement of the resources (16.67%), improvement of the teaching method (8.33%), and improvement of the program duration (8.33%).Global satisfaction was scored with the maximum value (5.00) by all the students.
Discussion & conclusions: Finding professionals specifically qualified and experienced for the generation of tissue engineered medicinal products and other ATMP is challenging, and very few training programs are available in Europe. Formation is crucial not only in technical aspects relatedto biofabrication, but also in regulatory aspects concerning generation, quality controls, release anduse of ATMP generated by tissue engineering. The present formative program has demonstrated to be very well evaluated by the students.
Acknowledgements: This work was supported by CTS-115 (Tissue Engineering Group).

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P86 - Influence of the recent COVID-19 pandemic on pre-university education of medical students

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Introduction: The World Health Organization (WHO) declared the coronavirus disease (COVID-19)a global pandemic on 11 March 2020. In addition to its immediate public health and socio-economicconsequences, the pandemic has also had a significant impact on global education [1]. The measures implemented to address the pandemic significantly altered the teaching-learning processof medical students [2]. However, there has been no in-depth research into whether these measures may have affected the formation of students who subsequently accessed to medical degrees. The objective of this study was to assess the academic performance of pre-COVID and post-COVID pre-college students enrolled in the first year of the Medicine degree at the Universityof Granada, in order to determine the impact of pandemic educational measures on students accessing a medical degree.
Methods: In this study, we analyzed the final examination scores of students enrolled in subject related to Cytology and Inheritance, which forms part of the first year of the Medicine degree at theUniversity of Granada, Spain. Scores were analyzed and compared among students correspondingto the last five academic courses (AC): students who received pre-COVID pre-univerisity education(AC19-20 and AC20-21), students with a mixed formative program (AC21-22) and post-COVID students (AC22-23 and AC23-24). The final examination test consisted of 60 multiple-choice questions, with 1 point awarded for a correct answer and -0.33 points for an incorrect answer. To determine the homogeneity of the five final examinations analyzed in this study, the Cronbach α coefficient was determined.
Results: Regarding consistency, the α coefficient showed a high reliability index in all AC, with, non-significant differences among the different tests. When the final examination scores were analyzed, results showed that the mean scores of pre-COVID students were significantly higher than those of post-COVID students (6.05 ± 1.73 vs. 5.35 ± 1.77, respectively). However, there wereno statistically significant differences between the pre-COVID students and mixed students. In addition, analysis of the percentage of items correctly answered showed a significantly higher percentage of correct items in pre-COVID students as compared to post-COVID students ($p<0.05$).Discussion & conclusions: The present study suggests that the post-pandemic teaching system may be associated with lower performance of the post-COVID students at the beginning of the Medicine degree, and that this reduction is mainly related to a lower percentage of unanswered questions. This may be associated to lower levels of confidence and greater aversion to uncertaintyamong post-COVID students. These findings should provide a basis for future educational interventions for post-COVID students to increase their self-confidence in order to improve their final performance.
Acknowledgements: Supported by CTS-115 (Tissue Engineering Group).

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P87 - Satisfaction results of medical students after the introduction of a new interactive Histological States Microscopic Image Bank

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Introduction: Historically, histology education has relied on traditional teaching methods such as lectures, laboratory sessions with microscopes, and textbooks featuring static images. However, as educational paradigms evolve in response to technological advancements and student preferences, there is a growing recognition of the need for innovative teaching approaches to enhance the learning experience. According to Heidger et al. (2002) [1], students exhibit notably enhanced learning outcomes through self-directed study with interactive histological slides via virtual microscopy compared to conventional methods reliant on static images from histological atlases. This shift enables students to develop structural histological identification skills more effectively, comprehensively, and integratively within a virtual microscopy framework.
Methods: The current study involved the creation of a Histological States Microscopic Image Bank(HSMIB), accessible autonomously by students enrolled in the first course of Medical Histology within the Medicine Degree program of the University of Granada, Spain. This resource provides students with the opportunity to access a comprehensive histological database, reinforcing key course concepts and actively engaging them in the learning process. Through guided dynamic histological images, students can identify the main structures discussed in practical sessions, while additional unguided images serve to strengthen previously further acquired theoretical competencies. After the implementation of this new interactive tool, medical students were asked to complete voluntarily and anonymously a satisfaction survey using the Lineker scale (1 to 5) evaluating ease of use, image quality, effectiveness in exploring histological preparations, impact on learning, and preference over conventional tools.
Results: The survey was completed by 118 students, being 69.5% female students, 28.8% male students and 1.7% students who did not identify with the previous options. HSMIB received high ratings for effectiveness in exploring histological preparations, with average scores exceeding 4.8 in all evaluated categories. Students reported improved understanding, correction of misconceptions, and a preference for HSMIB over conventional atlases. Access to annotated images facilitated remote studying, increased confidence in structure identification, and complemented theoretical and practical sessions effectively.
Discussion & conclusions: The Histological States Microscopic Image Bank (HSMIB) proved to bea highly effective educational resource in histology, noted for its user-friendly interface, excellent image quality, and precise annotations. It facilitated the sample exploration compared to using a traditional microscope, leading to enhanced comprehension and fostering a deeper interest in histology. Recommended for use by other histology students and proposed for inclusion inadvanced courses, HSMIB offers convenience and elevates the learning experience.
Acknowledgements: Supported by Proyectos de Innovación Docente y Buenas Prácticas del Plan FIDO UGR 2022-2023 (CÓDIGO 22-182), Unidad de Calidad e Innovación Docente y prospectiva, PlanPropio de la Universidad de Granada, and CTS-115 (Tissue Engineering Group), Junta de Andalucía, Spain.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P88 - Gamification or Learning Histology in Medicine

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Introduction: Teaching in the field of Medicine is a constantly evolving field open to newmethodologies. In contrast to a traditional lecture-based model, there is a push towards implementing strategies that emphasize active learning, which is considered one of the best methods for knowledge transmission and retention according to the literature. Gamification is defined as the use of game elements in an educational environment with the aim of enhancing student performance and motivation. The HistolWord activity, developed by the Department of Human Histology, uses gamification to promote teaching through group competition and the resolution of a series of questions related to the study content.
Objectives: To determine whether there is a positive assessment of student learning regarding participation in the HistolWord activity. Likewise, to study whether this strategy has a positive impact on academic performance.
Methods: Scientific article research was conducted through searches in various open-access publication repositories and databases. A descriptive statistical study was carried out by anonymous opinion surveys, with prior authorization from the students. Final grades from the last six academic years were also consulted to establish possible differences.
Results: The high participation of students in this activity is noteworthy. There is a positive impactof HistolWord on students' subjective assessment, in class attendance and particularly in areas related to improving motivation and its usefulness in preparing and reviewing course material. There are statistically significant differences ($p<0.03$) in the final grades of courses where the activity was implemented compared to those where it was not.
Conclusions: The use of gamification strategies, such as HistolWord, combined with the master class model, has shown multiple benefits for undergraduate medical students. Gamification shouldbe considered as a complement to traditional teaching within university curricula.

Histology and Histopathology
From Cell Biology to Tissue Engineering

P89 - Students' perceptions on seminar-based teaching in histology

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Introduction: Teaching in medical histology is based on different didactic modalities, such as theoretical, practical, and seminar-based teaching. Seminars contributes to integrate knowledge related to histological images [1]. In the Department of Histology of the Medical School of the University of Granada, students enrolled in the Degree in Medicine receive seminar-based teachingin which teachers present a clinical case with clinical and histological images for discussion by thestudents, who had to interpret and justify their possible diagnosis and treatments based on their own histological knowledge. In the present work, we have evaluated the students' perceptions on seminar-based teaching in medical histology.
Methods: An ad-hoc anonymous questionnaire was designed to evaluate the students' perceptionson their own experience during seminar-based teaching in histology. This questionnaire contained5 questions related to: 1) how useful has been the seminar to understand the histological conceptsstudied to the date in theoretical sessions? 2) how useful has been the seminar to understand thehistological preparations and images studied to the date in the practical sessions? 3) How useful was working on a clinical case during seminar-based teaching in histology? 4) How useful was working on the concepts previously studied in theoretical and practical sessions? 5) Do you think that the clinical case studied in the seminar sessions was properly related to the topics previously studied in theoretical and practical sessions?. Results were rated by the students using a Likert- like scale ranging from 1 (very bad) to 5 (excellent), and results were compared with students accessing the university via selective evaluation modality (selectividad) vs. students corresponding to other titles or degrees previously obtained using Mann-Whitney statistical tests. Specific improvement suggestions were also inquired in a open question.
Results: Results showed that the items with higher rates raised by the students was question 5,with an average score of 4.89±0.37, followed by question 3 (4.88±0.43) and question 2 (4.80±0.47),with the lowest scores assigned to question 1 (4.79±0.47) and question 4 (4.77±0.52). Interestingly,the item rated with the highest percentage of students assigning the maximum score (5) wasquestion 3 (91.4% of the students), whilst the item with the lower percentage of students rating thisitem with 5 was question 1 (80.9% of students). When results were compared between students corresponding to selective process were compared to other degrees, differences were non-significant ($p>0.05$) for the 5 questions analyzed. Specific comments were always very positive,and improvement actions suggested by the students are mainly related to the study of more typesof tissues, the use of additional histological images and the study of other types of clinical cases. Discussion & conclusions: In general, students rated very positively the use of seminars in medical histology. Students perceived seminar-based teaching as very useful to reinforce the histologicalknowledge that they previously had, especially regarding interpretation of histological images,without differences between both groups of students. In general, seminar-based teaching can beused as an useful didactic tool in medical histology.
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P90 - Assessing a dynamic learning approach in the practical teaching of Human Organography in the medical degree program.

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Introduction: The landscape of education is rapidly evolving, demanding a new and expansive skillset from educators. With digital devices and applications becoming omnipresent, educators must hone their digital proficiency. The European Framework for the Digital Competence of Educators [1] serves as a cornerstone, providing a comprehensive framework to cultivate educator-specific digital skills across Europe. It underscores the professional commitment of teachers to leverage digital resources in the teaching-learning process, fostering enhanced knowledge acquisition and active student engagement. This proactive approach to learning not only empowers students to take ownership of their educational journey but also molds their cognitive processes, shaping theirperspectives and actions. It instills a lifelong ethos of continuous learning, seamlessly integrating digital literacy into all facets of life [2]. At the forefront of this transformative strategy is the implementation of cutting-edge digital tools such as Wooclap. By leveraging this platform, educators elevate teaching standards, fuel knowledge acquisition with motivation, and cement student engagement with the theoretical underpinnings of their subjects. Methodology: Wooclap is a user-friendly tool designed for real-time interaction with our audience, enhancing student engagement. Its versatility allows for seamless integration into both traditional and online classes, whether synchronous or asynchronous. Operating via mobile phones, it captivates students by leveraging their devices as allies. Accessible through an alphanumeric codeon the web (www.wooclap.com) or by scanning a QR code, students can easily engage with the tool. Wooclap boasts various features, enabling interaction with the audience through multiple- choice questions, polls, open-ended questions, word clouds, numerical value questions, associations, image identification and more. Educators can tailor its use to meet their specific needs. In practical sessions, instructors provide students with histological samples related to the tissue or organ under study, elucidating practice objectives and guiding student observations whileaddressing any doubts regarding histological structure identities. In this particular experience, four groups of second-year Medicine students, comprising approximately 25-30 students each, enrolled in the subject of Human Organography, engaged in the observation and microscopic identificationof various preparations related to the digestive system. Following this, they anonymously responded to five image-based questions of different types (*label on image, multiple-choice and find on image*) and two final poll and rating questions to gauge satisfaction with the experience andassess knowledge acquired during the practical session. This initiative is part of an educational innovation project granted by UAH, titled “Dinamización digital del aprendizaje mediante inserción de Wooclap como Metodología Interactiva Docente II” (UAH/EV1377, 2022-23). Results: The most challenging type of questions for students was labeling structures on images, with only 45.02% of correct answers. It should be noted that this type of question requires studentsto identify three structures in the image and provide the correct name for each. Multiple-choice questions had a higher success rate, with 82.06% of students answering them correctly. The success rate increased further to 89.12% for questions that required finding structures on images.Regarding the students' experience with Wooclap, a significant majority (93.50%) expressed satisfaction with the platform. Additionally, 86.50% indicated they would recommend continuing touse Wooclap in the practical teaching of the subject. Discussion & conclusions: These findings suggest that while labeling structures on images provedto be the most challenging task for students, overall satisfaction with Wooclap was high, indicatingits effectiveness as a tool for practical teaching of the subject.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

P91 - Group dynamics in the classroom to promote the acquisition of theoretical knowledge in Cell Biology

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Introduction: Nowadays, there is a constant need to improve the quality of teaching in higher education. Teachers are making adaptations to traditional methods, giving great importance to the integration of participatory methodologies where students acquire a more active role in their training to improve the teaching-learning process. In this context, the integration of game elements in teaching and learning could motivate students, improve their participation and promote the development of skills and competencies relevant to their training [1]. In this way, the incorporation of game elements in teaching and learning can make academic content more interesting, attractive, and meaningful for university students [2]. Therefore, the objective of this teaching innovation proposal was to evaluate the usefulness of playful group dynamics in the classroom, to promote the acquisition of theoretical content and interest in the subject.
Methods: This teaching experience was performed during the second semester of the 2023-2024 academic year, with 74 students enrolled in the core subject Cellular Biology taught in the first year of the Degree in Marine Sciences at the University of Alicante. Prior to the activity, the teacher asked the students to review the theoretical content taught corresponding to the endomembrane system, vesicular transport, and secretory/endocytic pathways. These contents were chosen since they present greater complexity and memory difficulty. On the day of the classroom activity, an adaptation of the popular game Jenga was implemented. To do this, the students were divided into teams to face each other, taking turns to remove a block from the tower. Each removed block was placed on top of the tower, thus creating a progressively more unstable structure. The students had the option of asking the teacher a question related to the subject's syllabus. If the students got the question right, the turn passed to the opposing team and, if they got it wrong, they had to remove a block from the tower. The game ended when one team collapsed the tower.
Results: Once the activity was completed, in order to know if it had been useful for the acquisition of theoretical content, the students answered an opinion questionnaire. In general, the opinion of the students about the methodology used was very positive. Specifically, 93% of the students agreed or totally agreed that the theoretical contents chosen had been appropriate for the activity. Likewise, 96% of the learners agreed or totally agreed that the activity served to promote teamwork and create a good climate in the classroom. Furthermore, 93% of the students agreed or totally agreed that the activity had been useful to them to prepare for the theoretical exam and that they would recommend it as a complementary tool to traditional university teaching. In relation to the personal opinions written, the students generally appreciated this activity, highlighting that it was didactic and entertaining and that it helped break away from the routine of classes.
Discussion and conclusions: The integration of game elements and specifically this group dynamic of teaching innovation is an appropriate resource to promote the acquisition of theoretical content, encourage student participation and promote interest in the subject in the university environment.

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Conferences

Conference

De Ignacio Pirovano a Eduardo De Robertis. Origen y hechos evocables de la Histología argentina.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

From Ignacio Pirovano to Eduardo Diego Patricio De Robertis. Origin and evocable facts of Argentinean Histology

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The evocation's motivating and encouraging value guides this well-deserved tribute to the sesquicentennial of the Argentinean Histology, born at the Faculty (School) of Medicine of Buenos Aires in 1874. Such remembrance passes through two national consular figures of the Medicine: Professors Doctors Ignacio Pirovano (Buenos Aires 1844 – 1895) and Eduardo Diego Patricio De Robertis (Buenos Aires 1913 -1988), in addition to rescuing the critical predecessors that enabled De Robertis to raise such discipline to unquestionable excellence. As an inexorable result of a historical context, the trajectory mentioned above is framed and developed in parallel with the itinerary of the also sesquicentennial and award-winning Spanish Histology with its own epochal framework.

Conference

Sesquicentenario de la Sociedad Española de Histología e Ingeniería Tisular (SEHIT).

Eliseo Carrascal Marino.
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Manuel Garrosa García.
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Histology and Histopathology
From Cell Biology to Tissue Engineering

150 years since the origin of SEHIT

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The Spanish Society of Histology and Tissue Engineering (SEHIT) celebrates this year, 2024, the150th anniversary of the founding of its seminal predecessor, the Histological Society of Madrid, whose solemn inauguration took place on February 11, 1874. This scientifical society, also knownas Spanish Histological Society, was promoted by the first full professor of Histology in Spain, Aureliano Maestre-de San juan y Muñoz, who soon after his access to the chair of Normal and Pathological Histology of the Central University (today, Complutense University of Madrid) knew how to bring together other histologists to found the above mentioned society, with the aim to stimulate the development of this flourishing histological science through the creation of synergies,as well as promoting the provision of more Histology chairs in the rest of the universities in Spain. The new science of Histology was born in the mid-19th century, being considered the Handbook ofHuman Tissue Science by Kölliker (1852) the one that bases current fundamental concepts.

By that time, histologists in Spain were few and worked in Anatomy Departments, in some hospitalsand in some extra-academic laboratories. Among the latter we can mention the Free Spanish School of Medicine and Surgery founded by Pedro González de Velasco in his mansion- anthropological museum (many years later, Cajal's Laboratory of Biological Researches will occupyone of its wings); The Institute of Surgical Therapeutics, founded by Federico Rubio; The San Juande Dios Hospital in Madrid with its histological laboratory directed by José Eugenio Olavide; and The Royal Academy of Medicine of Madrid (later, RANME). Maestre was elected president of this new Society, which held seminars every week about histological topics, anatomic-clinical sessions, and theoretical debates, which still included passionate discussions between vitalism and mechanicism. The Society also provided practical activities in their three laboratories naming Histology, Experimental, and Histochemistry. After a few years, the Society disintegrated in 1877 to be continued in the section of Histology belonging to the Medical-Surgical Academy from whichit had mainly originated.

The Society was refounded in the time of "The Transition" in Spain, again promoted from the chairof Histology of the Faculty of Medicine of Complutense University of Madrid, whose full professor Luis Zamorano (7th successor of Maestre) got gather the histologists of all over the country to reestablish the Society on June 6, 1977, now with the name of Spanish Society of Histology, Prof.Zamorano being elected its president. The Society, then, took as its official journal the founded byProf. Lucio Díaz-Flores in Granada Morphología Normal y Patológica, Secc. A, which reached international prestige and played a fundamental role in the development of Histology in Spain up to 1983, when quit edditing, being substituted by the journal ACTA MICROSCOPICA edited by Prof. Jaime Merchán from Alicante until 1985. After that, the members of the Society received gratisthe prestigious journal Histology and Histopathology founded by Prof. Francisco Hernández in Murcia. Due to the rise of the therapeutic aspect of Histology through Tissue Engineering, the Society turned to be named in 2003 Spanish Society of Histology and Tissue Engineering. Since 1979, the Society has held a biennial meeting, first with the name of "National Congress of Histology", since 2001 "National Congress of Histology and Tissue Engineering, then going to international level in 2006, and since 2014 including the name of "Ibero-American Congress of Histology" in alternation with the Mexican Society of Histology. The years between the biennial meeting, a symposium named HistoDocencia is held to present advances on Histology and TissueEngineering teaching-learning processes and techniques.

The SEHIT has currently about 280 members including physicians, cell biologists, veterinarians, biotechnologists, dentists, pharmacists, chemists, biomedical engineers, and health biologists, as well as other related specialties in the biohealth field. It has implemented the new technologies, soit has a web site, a virtual forum, and virtual seminars as well as social media naming Facebook, Instagram and X, but maintaining the same spirit, enthusiasm and love for Histology as the pioneersin the 19th century founding the Society of which the SEHIT is heir.

Conference

Células M, la puerta de Ishtar al templo del GALT - brazo inductor de la inmunidad mucosa.

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Histology and Histopathology
From Cell Biology to Tissue Engineering

Membranous cells. The Ishtar gate to the GALT temple – inductor arm of the mucosal immunity.

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The gut mucosa exposes a huge area of 400 m2 where two necessities collision: the absorption of nutrients through a simple and vulnerable epithelium and the requirement of robust mechanisms of defense due to the rich local microbiota. To prevent the deleterious immune response of rejection against the great luminal content, two mechanisms have evolved. One of them is the exclusion, through the IgA production, resistant in a rich enzymatic milieu, anchored to the mucus and easily eliminated by the peristaltic movement. The remaining phenomenon is the immune-suppression exerted by T lymphocytes previously educated to produce an immune response of tolerance. These two altogether, determine the gut immuno-homeostasis based in a tripod integrated by the epithelium, the microbiota and the mucosal immune system (MIS), interacting each other permanently. Paradoxically, to ensure a suitable function of these three elements, is necessary that small quantities of the luminal content pass the mucosal barrier. Since the massive passage would be harmful, the transport is carried in localized anatomical sites like the Peyer's patches, the cecal appendix and the solitary lymphoid nodes of the human gut. These determined localizations constitute the immune-inductor arm of the mucosal immune system, also called GALT (gut associated lymphoid tissue), where the luminal content is monitored, translated and presented to the immune system ubicated below, to promote the IgA synthesis and stimulate the immune tolerance. The GALT is compared to the antique city of Babilon, since in synchrony with the thymus, appeared early in mammalian evolution, from the endoderm. GALTs are highly compartmentalized histo-architecturally, with a domus that protrudes directly to the lumen. Deeply lymph nodes are encountered where IgA synthesis is induced, in close proximity with T cells areas, where high endothelial venules are evidenced. The domus are covered by a simple epithelium called FAE (follicle associated epithelium), where M (membranous) cells are located; a unique phenotype specialized in the controlled traffic of the luminal content. In this regard, the M cells are analogous to the main portal in Babilon, the Ishtar gate. M cells determine membranous pockets where the lymphocytes rest. These immune cells, which enter to the intraepithelial space, are predominantly memory ones and have privilege because they are close to the luminal antigens and separated from the rest of the mucosal immune system. In the M cell pockets they acquire a lymphoblast morphology, activate mitosis, and from here they could extrude to the lumen. However, in a great extent, they direct to the mesenteric lymph nodes where the clones are expanded. Finally, they complete their path, colonizing the rest of the gut and the stroma of salivary and mammary glands, where complete their maturation and secrete IgA or exert the immune tolerance.

Conference

Historia y realidad de la Ingeniería Tisular en España (1999- 2024).

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Histology and Histopathology
From Cell Biology to Tissue Engineering

The institutionalization of tissue engineering in Spain (1999-2024)

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Histology was established as a scientific discipline in the 19th century. It is based on research and descriptive knowledge, using magnifying instruments, of the microscopic structures that make up living beings. In the first half of the 20th century, there was an important paradigm shift that interpreted tissues within the constructive framework of the different levels of organization that make up organisms. In the last decade of the century, in 1993, an important contribution was also made in this context by establishing the bases of the so-called tissue engineering, a set of processes that lead to obtaining artificial tissues with biomimetic characteristics to native tissues.

Institutionalization is the series of actions and procedures that allow something to be inserted into institutions, making it a permanent part of a community. The institutionalization of tissue engineering in Spain has been carried out over the last 25 years at various levels: epistemic and scientific community, academic-teaching, research and translation. At the epistemic level, it was provided with a solid conceptual substrate that inserts it into histology, a science whose goal is the study of tissues. The argument that supports this was published by the author of this paper in 2001 in the book "*New challenges for the Teaching and Research of Histology*" in the 21st Century [1], edited by the Mexican Society of Histology and in the texts published by the Royal National Academy of Medicine of Spain in 2004 and 2023 [2, 3]. At the level of the scientific community, the first contribution was made by the same author at the National Congress of the Spanish Society of Histology in 1999, a society that, as a pioneer, in 2001, agreed to change its name to become the Spanish Society of Histology and Tissue Engineering. At the academic-teaching level, the Faculty of Medicine of Granada incorporated tissue engineering as an optional subject in the degree of medicine in 2001. At present, this subject appears only in four other Faculties of Medicine and in 18 other degrees, from different universities, in the studies of Biomedicine, Pharmacy, Biotechnology and Biomedical Engineering. There are also 7 official master degrees in 7 Spanish universities. Regarding books, the Panamerican publisher text "*Histology and Oral Embryology*" incorporates tissue engineering in its title and content from its third edition in 2009. At the research level, the number of communications on tissue engineering at the congress of the scientific society increases from 1 in 2001 to 56 in 2022. The original articles produced in Spain and included in the international repertoires are 2,145 originals and 524 reviews. The indexed journal of the scientific society entitled "*Histology & Histopathology"*, initially subtitled "*Cellular and molecular biology*", was later subtitled *"From cell biology to Tissue engineering".* At the translation level, the Histology Department of the University of Granada stands out with 9 patents and two clinical trials (artificial cornea and palate) out of the five authorized by the Spanish Agency of Medicines. The other three, and by different groups, are carried out in Barcelona and Seville. Last June, the Agency approved as the first medicine generated by tissue engineering the artificial skin created by the Granada group called UGRSKIN.

In summary, it can be said that in Spain, and with regard to its institutionalization, tissue engineering is epistemologically embedded in histology and in its scientific society, it is being progressively incorporated into the academic world in medicine and health sciences, it is the object of relevant attention in research activity and it is also generating the translation of its products through patents and potential advanced therapies medicines. And this is only 30 years after the seminal article "*Tissue engineering*", by Robert Langer and Joseph Vacanti, published in *Science*, which is considered the formal beginning of tissue engineering as a field of knowledge.

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