



VI SIMPOSIO DE  
T E R A P I A S  
**AVANZADAS**  
TECNOLOGÍAS  
B I O M É D I C A S

FACULTAD DE MEDICINA DE LA UNIVERSIDAD DE GRANADA

**28 NOVIEMBRE 2025**

SALÓN DE ACTOS

## INFORMACIÓN GENERAL

El Área de Terapias Avanzadas y Tecnologías Biomédicas del instituto de Investigación Biosanitaria ibs.GRANADA organiza el **VI SIMPOSIO DE TERAPIAS AVANZADAS Y TECNOLOGÍAS BIOMÉDICAS**. Tendrá lugar el viernes **28 de noviembre de 2025** en el Salón de Actos la Facultad de Medicina de la Universidad de Granada.

## OBJETIVOS

El **objetivo** de este simposio es presentar las principales líneas de investigación llevadas a cabo en el área de las Terapias Avanzadas y las Tecnologías Biomédicas por grupos del ibs.GRANADA y grupos afines a estas áreas de conocimiento. El simposio supone una oportunidad para que investigadores y profesionales sanitarios puedan interaccionar y establecer nuevas líneas de colaboración y sinergias en el campo de las Terapias Avanzadas y las Tecnologías Biomédicas. Este simposio es además una oportunidad para difundir estos trabajos y proyectos al resto de profesionales sanitarios, así como al público en general.

## INSCRIPCIONES

La **asistencia** es libre y gratuita con previa inscripción a través del siguiente enlace:

<https://www.ibsgranada.es/inscripcion-simposio-terapias-avanzadas-tecnologias-biosanitarias-ibs-granada/>

**El plazo límite para realizar la inscripción es el 27 de noviembre de 2025.**

## COMITÉ ORGANIZADOR

**Jesús Chato Astrain.** Prof. Departamento de Histología, Facultad de Medicina, Universidad de Granada. TEC03-Ingeniería Tisular.

**Óscar Darío García García.** Prof. Departamento de Histología, Facultad de Medicina, Universidad de Granada. TEC03-Ingeniería Tisular.

**Francisco Javier Molina Estévez.** Investigador del Departamento de Biología Celular. TEC24-Grupo de Investigación en Hematología y Terapia Génica: HemaTerGe.

## COMITÉ CIENTÍFICO

Araceli Aguilar González  
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Paula Ávila Fernández

El programa y libro de Abstracts del VI Simposio de Terapias Avanzadas y Tecnologías Biomédicas del ibs.GRANADA se encuentra públicamente disponible en la web del ibs.GRANADA y el en repositorio europeo Zenodo (<https://zenodo.org/>).



## PROGRAMA

**08:30-09:00h** Reunión de coordinación de los investigadores responsables del Área de Terapias Avanzadas y Tecnologías Biomédicas

**09:00-09:20h** Inauguración del Simposio

- **Dr. José Juan Jiménez Moleón.** Decano de la Facultad de Medicina. Universidad de Granada
- **Dr. Indalecio Sánchez-Montesinos García.** Delegado territorial de la Consejería de Salud y Consumo de la Junta de Andalucía
- **Dra. María Ángeles García Rescalvo.** Directora Gerente del Universitario Virgen de las Nieves
- **Dr. Manuel Enrique Reyes Nadal.** Director Gerente del Hospital Universitario Clínico San Cecilio
- **Dra. María José Sánchez Pérez.** Directora Científica de ibs.GRANADA
- **Dr. Óscar Darío García García.** Organizador del VI Simposio de Terapias Avanzadas y Tecnologías Biomédicas.

**09:20-09:40h** Conferencia Invitada: PV-Skin: Un sustituto de piel con cuatro tipos celulares para grandes quemados

**Dra. Rocío García de la Cruz Valencia**

*Translational Research Manager, Kinderspital Zürich, Suiza*

**09:40-10:55h** Mesa 1: Terapias avanzadas: Ingeniería Tisular

- **Exosomas derivados de células madre mesenquimales en regeneración tisular.**  
*D. Víctor Javier Costela Ruz. Investigador del grupo TEC17 – BIOTEJSALUD ibs.GRANADA*
- **Comunicación mediada por vesículas extracelulares entre plaquetas y células tumorales: rol en la carcinogénesis y trombosis en cáncer de páncreas (Estudio placetro)**  
*D. Antonio Palomeque Jiménez. Investigador del grupo TEC13-Cirugía Avanzada ibs.GRANADA*

- **Skin Cancer Model platform for drug testing**  
*Dña. Paula Ávila Fernández. Investigadora del grupo TEC03 – Ingeniería Tisular ibs.GRANADA*
- **In vitro reconstituted ECMs for personalized tumor modelling**  
*Dña. Julia López de Andrés. Investigadora del grupo Grupo: TEC16 – Terapias Avanzadas: Diferenciación, Regeneración y Cáncer ibs.GRANADA*
- **Diseño y Caracterización de Hidrogeles Multisensibles basados en Quitosano, Agarosa y Partículas Magnéticas**  
*D. Modesto Torcuato López López. Investigador del grupo Grupo TEC05-Física de Interfases y Sistemas Coloidales ibs.GRANADA*

### **10:55- 11:40 h Mesa 2: Terapias Avanzadas: Terapia Génica y Farmacogenética**

- **Advanced gene editing applied to safer gene therapy and novel Pompe disease models**  
*D. Francisco Javier Molina Estévez. Investigador del grupo Grupo: TEC24-Grupo de Investigación en Hematología y Terapia Génica: HemaTerGe ibs.GRANADA*
- **Dual-CRISPR Systems: Advancing Genome Editing and Diagnostics**  
*Dña. Araceli Aguilar González. Investigadora del grupo TECE18 – NANOCEMBIO ibs.GRANADA*
- **Práctica Segura en el Uso de Medicamentos: Integración de la Farmacogenética con GenHUSC App y Desconstrucción de la Vacuna de ARNm-NPLs**  
*D. Xando Díaz Villamarín y Pilar Baena Álvarez. Investigadores del grupo TEC01 – Práctica segura en el uso de medicamentos ibs.GRANADA*

### **11:40-12:20h Café y visita a pósteres**

### **12:20-12:30h Conferencia Patrocinada: Innovación tecnológica en Biología Celular y Terapias Avanzadas**

**D. Aitor González Granja**  
*Marketing Manager at Izasa by Palex*

### **12:30- 13:30 h** Mesa 3: Salud y Rehabilitación: Estrategias Clínicas y Tecnológicas

- **Asociación de cambios en la materia gris con los resultados funcionales y de calidad de vida en pacientes con ictus tras cirugía de la extremidad superior espástica. Resultados a seis meses de un ensayo aleatorizado**

*Dña. Patricia Hurtado Olmo. Investigadora del grupo Grupo: TECE21 – Reparación, Regeneración y Sustitución Ósea ibs.GRANADA*

- **Intervención multimodal en dolor lumbar crónico**

*D. Víctor Segura-Jiménez. Investigador del grupo TECE20 – REHABILITA-T: Avances e Innovación en Rehabilitación y Promoción de la Salud ibs.GRANADA*

- **Inflamación y riesgo cardiovascular en psoriasis e hidradenitis**

*D. Manuel Sánchez Díaz. Investigador del grupo TECE19 – Dermatología Clínica y Traslacional ibs.GRANADA*

- **Avances en enfermedad renal hereditaria**

*D. Rafael J Esteban De la Rosa. Investigador del grupo TEC14 – Reproducción Humana y Enfermedades Hereditarias y Complejas ibs.GRANADA*

### **13:30-14:15h** Mesa 4: Imagen Médica y Modelado Computacional

- **PET/TC en inflamación vascular en hipercolesterolemia familiar**

*Dña. María E. Bellón Guardia. Investigadora del grupo TEC15 – Medicina Nuclear y Molecular ibs.GRANADA*

- **Modelado computacional del color dental**

*Dña. María Navidad Tejada Casado. Investigadora del grupo TEC09 – Óptica de Biomateriales y Tejidos ibs.GRANADA*

- **Algoritmo Cervisense TPTL para predicción de parto prematuro**

*Dña. Paqui Molina. Investigadora del grupo TEC12 – Salud Materno Fetal y Elastografía ibs.GRANADA*

### **14:15h** Cierre de la jornada y conclusiones

VI SIMPOSIO DE

TERAPIAS  
**AVANZADAS**



**TECNOLOGÍAS**  
BIOMÉDICAS

LIBRO DE COMUNICACIONES

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COMUNICACIONES PRESENTADAS EN EL SIMPOSIO  
ABSTRACTS

## *Integración de la inteligencia artificial para fortalecer la precisión del diagnóstico histopatológico en cáncer de próstata*

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### TEC03-Ingeniería Tisular

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**Introducción:** El adenocarcinoma de próstata (CaP) es uno de los cánceres más frecuentes en hombres y constituye un desafío para la salud pública. La detección temprana es esencial para mejorar el pronóstico. En este contexto, la inteligencia artificial (IA) aplicada al análisis histológico surge como una herramienta prometedora para aumentar la precisión diagnóstica y distinguir el CaP de otras condiciones como la Hiperplasia Prostática Benigna (HPB).

**Objetivo:** Desarrollar y evaluar un modelo de IA basado en redes neuronales convolucionales (CNN) para el análisis automatizado de imágenes histológicas de CaP, con el fin de mejorar la exactitud diagnóstica y facilitar la diferenciación entre CaP y HPB.

**Materiales y Métodos:** Se llevó a cabo un estudio experimental empleando ResNet50 para la clasificación y U-Net para la segmentación de tejido prostático. La muestra estuvo compuesta por tejidos histológicos provenientes de pacientes diagnosticados con CaP. El estudio cuenta con aprobación de un Comité de Ética en Investigación y con el consentimiento informado de todos los participantes, cumpliendo con las normativas éticas vigentes. Las imágenes fueron sometidas a procesos de normalización de tinciones para estandarizar la variabilidad cromática y optimizar el análisis computacional.

**Resultados:** Los modelos desarrollados mostraron un rendimiento sólido en la segmentación de áreas tumorales. La normalización de tinciones contribuyó de manera significativa a mejorar la precisión del algoritmo, facilitando la distinción entre CaP y HPB. El sistema basado en IA logró identificar con elevada fiabilidad las regiones neoplásicas, proporcionando una evaluación más precisa del tejido.

**Discusión y Conclusiones:** Los hallazgos sugieren que la integración de IA con técnicas de normalización de tinciones potencia notablemente la capacidad diagnóstica en CaP. Este trabajo representa un avance relevante hacia el perfeccionamiento de herramientas automatizadas que apoyen el diagnóstico histopatológico, ofreciendo un enfoque prometedor para incrementar la exactitud en la identificación de esta enfermedad.

**Agradecimientos:** Proyecto Departamento de Histología, Universidad de Granada (UGR)-España.

PIEM 2024-Cod:2404 Proyecto de Investigación Escuela de Medicina, Universidad de Valparaíso (UV)-Chile.

Becas Chile -Doctorado en el extranjero (ANID).



*Adhesive performance of novel nanoparticle formulations doped with dexamethasone and doxycycline at the dentin–resin interface*

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TEC03-Ingeniería Tisular

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**Objective:** To synthesize several formulations of nanoparticles (NPs) doped with dexamethasone (Dexa) and doxycycline (Doxy) according to a Response Surface Modeling (RSM)-based experimental design and to assess the microtensile bond strength (MTBS) of the different formulations at the dentin–resin interface at 24 hours.

**Material and Methods:** Seven formulations groups, with different concentration of Dexa vs Doxy, were proposed four 24h after RSM. Dentin surfaces of third molars were divided into groups based on NP formulation and treated with NPs. Following a standard adhesive protocol, samples were stored in SBFS for 24 hours. Adhesion strength was assessed via MTBS and a fractographic analysis of the fractured interface was performed, analyzed statistically using ANOVA and Tukey test ( $p < 0.05$ ).

**Results:** The mean values and standard deviation of MTBS obtained for the experimental groups are shown in the Figure.

**Abbreviations:** dexamethasone (Dexa) and doxycycline (Doxy) MTBS: microtensile bond strength to dentin; MPa: Megapascals; SBFS: simulated body fluid solution. Same letter indicates no significant differences between treatment groups ( $p < 0.05$ ).

**Conclusions**

Doxycycline incorporated into SBFS-based formulations, either alone or in combination with dexamethasone, exhibited enhanced dentin bond strength relative to the other experimental groups

**Acknowledgements**

Grant C-CTS-189-UGR23 funded by Consejería de Universidad, Investigación e Innovación and by ERDF Andalusia Program 2021-2027. H.L.B (P5B-2024-092) holds Research Fellowships for Master Students from the University of Granada.

*Green Synthesis and Characterization of Icariin-Loaded Beeswax Solid Lipid Nanoparticles for Innovative Dental Therapies*

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TEC03-Ingeniería Tisular

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**Objective:** This study aims to synthesize and characterize icariin-loaded beeswax solid lipid nanoparticles, assessing their physicochemical properties.

**Material and Methods:** Solid lipid nanoparticles (SLNPs) loaded with icariin were synthesized by high-shear homogenization method and characterized using Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM), and Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) analyses.

**Results:** TEM images showed that SLNPs had a spherical shape. DLS analysis indicated an average particle size between 200 and 245 nm, and the polydispersity and zeta potential suggested that the particles were homogeneous and did not tend to agglomerate. The ATR-FTIR spectrum verified the presence of beeswax and icariin in their composition. **Conclusion:** This study successfully synthesized and characterized icariin-loaded beeswax solid lipid nanoparticles with favorable physicochemical properties. The nanoparticles demonstrated suitable size, morphology, and stability, ensuring efficient encapsulation of icariin. These promising results highlight the potential of green-synthesized lipid nanoparticles as effective and targeted drug delivery systems for dental applications

*Del carbón activado a las terapias dirigidas: materiales magneto-activos para liberación controlada y tratamiento localizado*

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**TEC05-Física de Interfases y Sistemas Coloidales**

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Las terapias avanzadas requieren sistemas capaces de liberar fármacos de forma localizada y controlada. Las partículas de carbón activado magnéticas (MAC) combinan la alta porosidad del carbón activado con la respuesta magnética y fototérmica de la magnetita, permitiendo modular la liberación mediante estímulos externos. Este trabajo evalúa su potencial como vehículos para el metotrexato (MTX), proponiendo que campos magnéticos rotatorios y radiación infrarroja pueden activar y acelerar la liberación del fármaco, ofreciendo una plataforma versátil para terapias dirigidas.

Las MAC se obtuvieron por deposición de magnetita sobre carbón activado mesoporoso (YP50F) y posterior recubrimiento con polietilenimina (PEI) para facilitar la adsorción de MTX. Su caracterización se realizó mediante microscopía electrónica, espectroscopía FTIR, magnetometría y medidas de potencial  $\zeta$ . La respuesta a estímulos se analizó aplicando campos magnéticos rotatorios y radiación láser infrarroja, evaluando la liberación por espectrofotometría UV-Vis. La biocompatibilidad se estudió en fibroblastos humanos mediante ensayos de viabilidad celular.

Las partículas mostraron comportamiento superparamagnético, confirmando la correcta incorporación de magnetita. El recubrimiento con PEI mejoró la estabilidad coloidal y la carga de MTX. Bajo estímulos externos, la liberación se duplicó respecto a condiciones pasivas, evidenciando un control eficaz de la cinética. La irradiación infrarroja generó incrementos térmicos moderados con tasas de absorción específicas cercanas a 180 W/g, validando su potencial fototérmico. Los ensayos de citotoxicidad mostraron viabilidades superiores al 85 % hasta 700  $\mu\text{g/mL}$ , confirmando su baja toxicidad.

En conjunto, las MAC se perfilan como plataformas versátiles para terapias combinadas de liberación controlada y tratamiento fototérmico, ofreciendo un enfoque prometedor para el desarrollo de sistemas activables de forma remota en el ámbito de las terapias avanzadas.

*Construcción y visualización de un espacio cromático dental difuso  
mediante espacios conceptuales: PyFCS*

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**TEC09-Óptica de Biomateriales y Tejidos**

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Desarrollar un espacio cromático dental continuo y basado en la percepción humana, utilizando la teoría de los espacios conceptuales y modelos difusos, que ofrezca una representación flexible e interpretable del color, y que permita reflejar la continuidad tonal del color dental y superar las limitaciones de los métodos discretos utilizados en odontología restauradora.

El sistema se desarrolló con PyFCS, una biblioteca de código abierto en Python para definir, manipular y visualizar espacios conceptuales difusos, especialmente en dominios perceptuales como el color. Permite construir categorías cromáticas mediante particionamiento tipo Voronoi, definir funciones de pertenencia y analizar imágenes. En este estudio se procesaron 16 muestras de la guía VITA Classical capturadas con cámara hiperespectral (DataColor), extrayendo valores cromáticos representativos en un espacio perceptual continuo. Se definieron funciones de pertenencia para cada tono y se construyó un conjunto difuso que modela su estructura interna. También se generaron visualizaciones 3D interactivas para explorar la geometría cromática, proximidades entre categorías y continuidad tonal.

El espacio cromático difuso mostró una organización coherente con la percepción clínica del color dental, revelando transiciones graduales, solapamientos perceptuales y proximidades cromáticas relevantes. Las visualizaciones tridimensionales evidenciaron gradientes y relaciones internas no visibles en modelos discretos, obtenidas directamente de imágenes procesadas con PyFCS, facilitando un análisis detallado de la variabilidad tonal.

Los espacios conceptuales difusos son una herramienta sólida y alineada con la percepción humana para representar el color dental. La integración de medidas hiperespectrales de DataColor en PyFCS permite construir espacios reproducibles, ampliables y adaptables a distintas guías clínicas, reforzando la coherencia entre datos instrumentales y percepción visual. Este enfoque sienta bases para sistemas avanzados de selección digital de color, optimización de protocolos clínicos y apoyo al diagnóstico cromático en odontología restauradora y estética.



## *DOES THE CHROMATIC ABILITY OF ONE-SHADED RESIN-BASED COMPOSITES LIMITED BY THE SURROUNDING? PRELIMINARY RESULTS*

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### TEC09-Óptica de Biomateriales y Tejidos

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One-shade composite resins are innovative biomaterials used in restorative dentistry that simplify dental shade selection due to their color-matching ability. This study evaluates the color adjustment potential of different one-shade composite resins.

Cylindrical single samples (15 mm diameter and 6 mm thick) of conventional composite (Vitapan Excell®) in three shades (A2, B2, C4) (n=27) and, of one-shade materials (Omnichroma n=3 and Omnicroma Flow n=3) were manufactured. Then, 18 samples in A2, B2 and C4 shades were restored with Omnicroma (n=9) and Omnicroma Flow (n=9), creating dual samples. The color of all single and dual samples was measured separately and under restoration conditions.

Color differences CIEDE2000 ( $\Delta E_{00}$ ) were analysed between combinations of conventional and one-shade materials, on both type of samples. Color adaptation potential (CAP00) was used to analyze the chromatic adaptation capability. Statistical analysis was performed using Kruskal-Wallis and Mann-Whitney test with Bonferroni correction ( $p < 0.0001$ ). Significant differences in CAP00 ( $p < 0.0001$ ) were found depending on the shade of the surrounding material, with lower performance in darker shades (C4). Significant differences were also found between one-shade materials ( $p < 0.0001$ ), showing Omnicroma Flow a higher color adaptation ability across all shades and giving its best results on light shades. On the other hand, conventional Omnicroma obtained better results on B2 than on A2.

In conclusion, within the limitations of our study, it can be stated that Omnicroma Flow performs better CAP00 than its conventional version, especially in lighter shades. Overall, one-shade materials exhibit lower color adaptation in darker shades.

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The authors declare that they have no conflicts of interest.

## *Herramienta basada en inteligencia artificial para estimar el fracaso en la extracción testicular de espermatozoides en pacientes con azoospermia no obstructiva*

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### TEC14-Reproducción Humana y Enfermedades Hereditarias y Complejas

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La azoospermia no obstructiva (NOA), debida a un fallo grave de la espermatogénesis (SPGF), se caracteriza por la ausencia de espermatozoides en el eyaculado. Algunos pacientes pueden obtener espermatozoides mediante su extracción testicular (TESE), aunque esta técnica suele fracasar en aquellos con el síndrome de solo células de Sertoli (SCO, ausencia de línea germinal). Dado que el diagnóstico de SCO requiere de la biopsia testicular, se necesitan herramientas no invasivas que anticipen el resultado de la TESE. El objetivo fue desarrollar un modelo de inteligencia artificial basado en biomarcadores genéticos y hormonales para predecir SCO y, por tanto, el fracaso de la TESE en pacientes con NOA.

Se incluyeron 293 varones infértiles con SPGF: 143 con SCO y 150 con otros fenotipos de NOA distintos a SCO. Se compararon tres modelos de machine learning (red neuronal artificial, bosque aleatorio y regresión logística) para predecir el fenotipo SCO. Los modelos incluyeron cuatro variables: (1) puntuaciones de riesgo poligénico asociadas a autoinmunidad, (2) presencia de un aminoácido específico en la proteína HLA-DRβ1, (3) niveles de hormona foliculoestimulante (FSH) y (4) ratio FSH/ hormona luteinizante (LH). El rendimiento se evaluó mediante métricas estándar en conjuntos de entrenamiento y test.

La red neuronal mostró el mejor rendimiento (AUC=0,65; precisión=0,61; sensibilidad=0,62; especificidad=0,61). La presencia de serina en la molécula HLA-DRβ1 fue el predictor más relevante, reforzando la hipótesis de una base inmunológica en SCO y de la asociación de la región del HLA de clase II con este fenotipo. Las puntuaciones de riesgo poligénico de autoinmunidad fueron significativamente superiores en pacientes con SCO, y tanto la FSH como la ratio FSH/LH contribuyeron a la predicción, en concordancia con estudios previos que asocian altos niveles en estas hormonas con infertilidad. Aunque prometedor, el modelo requiere ampliación muestral y adición de nuevos biomarcadores para mejorar su robustez. Esta aproximación podría ofrecer una estimación no invasiva del resultado de la TESE y evitar procedimientos innecesarios en pacientes con alta probabilidad de fracaso.

## *Caracterización multiómica de células Sertoli adultas como base para entender la disfunción reproductiva masculina*

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Los trastornos del desarrollo sexual (DSD) constituyen un grupo complejo de condiciones con alteraciones en la diferenciación de gónadas o genitales. La displasia campomélica (DC) es una de las formas más graves, asociada a mutaciones en SOX9, un factor de transcripción esencial para la formación esquelética y la diferenciación de células Sertoli, cuya disfunción causa fallo testicular y ambigüedad sexual. Aunque se conocen los efectos fenotípicos de la DC, aún se desconoce cómo se alteran los mecanismos que regulan la diferenciación de Sertoli. Nuestra hipótesis es que la caracterización multiómica single-cell de estas células permitirá definir estados y trayectorias cuya disrupción explica la fisiopatología de DSD como la DC.

Se analizó tejido testicular en ratones control mediante tecnología single-cell multiomics (10x Genomics Multiome), que combina transcriptómica (RNA-seq) y epigenómica (ATAC-seq) en células individuales. Se secuenciaron 1,511 células, detectando 33,697 genes y 76,603 picos de accesibilidad cromatínica. El clustering bioinformático permitió identificar subpoblaciones de células Sertoli y definir etapas funcionales y de diferenciación, complementadas con análisis de genes reguladores. La trayectoria celular se reconstruyó con algoritmos de RNA velocity y Monocle3. Se validó la especificidad comparando perfiles epigenómicos con referencias de Sertoli y granulosa. Métodos estadísticos robustos permitieron explorar la correspondencia genotipo-fenotipo y posibles impactos de mutaciones DSD.

Se identificaron 774 células de Sertoli en cuatro clusters reflejando un gradiente de diferenciación: inmaduras (SOX9<sup>++</sup>, TCF7L2<sup>+</sup>), intermedias (AMH<sup>+</sup>, CYP11A1<sup>+</sup>) y especializadas (KL<sup>+</sup>, EGF<sup>+</sup>). El análisis epigenómico mostró 83.1% de conservación con células de Sertoli de referencia y 68.2% de similitud con células de granulosa ovárica, sugiriendo mecanismos compartidos en determinación gonadal. Genes maestros como SOX9, GATA4 y factores meióticos se enriquecen diferencialmente entre subpoblaciones, identificando etapas vulnerables a mutaciones asociadas a DSD. Las trayectorias celulares por Monocle3 y RNA velocity presentaron 94% de concordancia, creando un atlas para proyectar perturbaciones génicas. Estos hallazgos permiten mapear correlaciones genotipo-fenotipo y definen marcadores moleculares, mostrando que la integración multiómica explica cómo mutaciones como SOX9 generan el espectro de DC y otros DSD, abriendo la vía a diagnósticos y terapias personalizadas.

## *Unveiling the immune landscape of Sertoli cell-only testes through single-cell transcriptomics in non-obstructive azoospermia*

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### TEC14-Reproducción Humana y Enfermedades Hereditarias y Complejas

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The human testis maintains an immune-privileged state that protects germ cells while hosting specialised immune populations. In non-obstructive azoospermia (NOA), particularly in the Sertoli cell-only (SCO) phenotype, this balance appears altered. Genetic associations with HLA class II molecules suggest that immune dysregulation may underlie SCO development, yet the specific immune mechanisms remain unclear. We hypothesised that SCO is driven by altered immune regulation leading to local inflammation and loss of immune privilege. To test this, we used single-cell RNA sequencing (scRNA-seq) to characterise immune and somatic landscapes in NOA testes and identify pathways linked to immune activation in SCO.

Testicular biopsies from fourteen NOA patients were analysed, including five with SCO and eight with other histological subtypes. Single-cell suspensions were processed using the 10x Genomics Next GEM platform, generating more than 80,000 single-cell transcriptomes after quality control. Data were integrated and analysed in Scanpy to identify cell clusters and define germ, somatic, and immune populations. Comparative analyses between SCO and non-SCO samples in terms of immune cell enrichment, activation states, and differentially expressed genes were performed.

Clustering analyses identified over twenty cellular clusters, including novel immune subpopulations. SCO testes showed marked enrichment of immune cells, notably pro-inflammatory CD8<sup>+</sup> T cells and Type 17 helper T cells, indicating increased infiltration and activation. Gene expression patterns reflected effector and inflammatory responses, suggesting a breakdown of testicular immune privilege. These findings support an immune-mediated component in SCO pathogenesis and highlight immune dysregulation as a key factor in severe NOA, offering new perspectives for diagnosis and immunomodulatory therapies in male infertility.



## *Single-cell transcriptomic profiling uncovers seminal cellular diversity in non-obstructive azoospermia*

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### TEC14-Reproducción Humana y Enfermedades Hereditarias y Complejas

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Non-obstructive azoospermia (NOA), a severe form of male infertility, is defined by absent sperm in semen due to testicular failure. Based on testicular histology, NOA can present as hypospermatogenesis (HS), maturation arrest (MA), or Sertoli cell-only syndrome (SCO), each with unique testicular cellular compositions and fertility prognoses. Despite advances in assisted reproduction, predicting testicular sperm extraction (TESE) success remains limited. This study uses single-cell RNA sequencing (scRNA-seq) to profile and compare cell populations in semen from different NOA subtypes, aiming to uncover microenvironmental differences between NOA subtypes and to support development of non-invasive predictors for TESE outcomes.

scRNA-seq technology was used to profile the transcriptomes of seminal cells collected from 16 Spanish men with NOA, classified as SCO (n=6), MA (n=4), or HS (n=6) based on testicular histology. Clinical TESE outcomes were collected post-biopsy to study links between TESE success and transcriptomic profiles. scRNA-seq libraries were generated using the 10x Genomics Chromium NextGEM platform, allowing high-resolution analysis of cell diversity across NOA subtypes and TESE outcomes. Following quality control, 35,646 single-cell transcriptomes were analyzed and clustered. Cell-type composition differences between TESE outcomes were assessed using permutational multivariate analysis of variance (PERMANOVA). All procedures received ethics approval and informed consent.

scRNA-seq analysis of seminal cells from NOA patients revealed marked cellular heterogeneity, identifying germ cells, immune cells, and non-immunological somatic cells. The immune fraction consisted of myeloid (macrophages, monocytes, dendritic cells) and lymphoid cells (B, CD4<sup>+</sup>, CD8<sup>+</sup> T, NK, NKT), indicating a complex local immune environment. Somatic cells included prostatic stem and epithelial cells; while germ cells were rare, in line with spermatogenic impairment. Notably, TESE- samples were enriched in macrophages and monocytes, while TESE+ samples contained more prostatic stem, epithelial, and germ cells (PERMANOVA,  $p=2.63 \times 10^{-2}$ ). These cellular signatures may predict TESE outcomes and improve candidate selection for TESE, potentially sparing patients from unnecessary surgical interventions.

## *Genetic Evidence from Mendelian Randomization Supports a Causal Role of Immune-Mediated Diseases in Severe Male Infertility*

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### TEC14-Reproducción Humana y Enfermedades Hereditarias y Complejas

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Genome-wide association studies (GWAS) have implicated the major histocompatibility complex (MHC) class II region in the genetic susceptibility to Sertoli cell-only (SCO) syndrome, a severe form of non-obstructive azoospermia (NOA) characterized by a complete absence of germ cells due to profound spermatogenic failure (SPGF). Given the immune-related genetic background of SCO, we employed a Mendelian randomization (MR) approach to explore potential causal links between autoimmune diseases and SCO, aiming to clarify the immune-mediated mechanisms underlying this extreme form of male infertility.

We evaluated immune-mediated traits as potential causal factors for NOA using publicly available GWAS results. The NOA dataset included 1,274 men with SPGF, comprising 502 SCO cases. Causal inference was performed through two-sample Mendelian randomization (2SMR) using the TwoSampleMR R package. Three complementary methods were applied: random-effects inverse variance weighted (IVW), weighted median, and MR-Egger regression. Genetic variants were selected as instrumental variables at genome-wide suggestive significance ( $P = 5 \times 10^{-5}$ ) and validated for strength ( $F > 10$ ). Sensitivity analyses assessed pleiotropy via the MR-Egger intercept and heterogeneity using Cochran's Q test. Causal effects were deemed significant at  $P \leq 0.05$ .

Using the IVW method, significant causal associations were identified between SCO and four immune-mediated traits: general autoimmune conditions ( $P=3.45 \times 10^{-2}$ ), celiac disease ( $P=7 \times 10^{-3}$ ), inflammatory bowel disease ( $P=4.58 \times 10^{-2}$ ), and Crohn's disease ( $P=9 \times 10^{-4}$ ). The positive causal relation between them indicates that a higher genetic predisposition to immune-related disorders increases the risk of SCO, supporting an immune-mediated genetic etiology. The overlap between epithelial barrier dysfunction in celiac and Crohn's diseases and the blood-testis barrier impairment in SCO could suggest a shared mechanism where barrier disruption exposes germ cells to immune-mediated damage. These findings support an immune-mediated genetic basis for SCO. Identifying markers of immune dysfunction may help predict unfavorable testicular sperm extraction outcomes in these patients, highlighting the clinical relevance of immune pathways for personalized counseling and management of severe male infertility.

## *Advancing CSC-Targeted Approaches in PDAC Using a Reproducible Preclinical Model of Liver Metastasis*

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### TEC16-Terapias Avanzadas: Diferenciación, Regeneración y Cáncer

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Pancreatic Cancer(PC) is a multifactorial disease with various endocrine and non-endocrine subtypes, the most common and aggressive being pancreatic ductal adenocarcinoma(PDAC), accounting for over 92% of cases. More than 50% of PDAC patients present metastasis in the liver, making >70% inoperable. This late diagnosis results in a 5-year overall survival rate of only 2% for metastatic PC(mPC), with a median survival of one year. Available treatments are limited to chemotherapy and palliative care. Metastasis requires tumor cells with self-renewal, plasticity, dormancy, and differentiation capabilities—key traits of cancer stem cells(CSC). Given these properties, CSC involvement in metastasis is likely. Here, we analyze CSC impact on metastatic PDAC(mPDAC) to the liver.

We selected a batch of four PDAC cell lines (ASPC-1, MIAPaCa-2, BxPC-3 and PANC-1) with varying levels of aggressiveness and distinct genetic profiles to better simulate the diversity observed in human patients. For tracking purposes, all cell lines stably expressed a luciferase reporter. Following a well-established protocol, CSCs were generated from the 2D cell lines and subsequently characterized by flow cytometry. Different cell concentrations were then selected for intrasplenic injections, followed by splenectomy to properly develop liver metastasis. Initial analysis and characterization were performed using quantitative PCR, flow cytometry, and immunohistochemistry.

In vitro, CSCs derived from PDAC cell lines exhibited distinct expression profiles of CD44v6, CD24 and ALDH1 confirming their heterogeneous stemness and metastatic potential. In vivo, ASPC-1-derived CSCs demonstrated the highest liver metastatic capacity compared to PANC-1, MIAPaCa-2, and BxPC-3, correlating with their aggressiveness and molecular phenotype. Detailed phenotyping of metastatic lesions revealed differential expression of CD44v6, Vim, CXCR4, and ALDH1, underscoring the relevance of CSC plasticity in driving metastatic adaptation and disease progression. Collectively, these results validate the robustness of our intrasplenic injection model to faithfully reproduce PDAC liver metastasis and provide a functional framework to investigate CSC-driven mechanisms. This model represents a valuable preclinical platform for identifying novel antimetastatic targets and developing CSC-focused precision therapies to improve outcomes in metastatic PDAC.

## *Impact of prevalent KRAS mutations in PDAC resistance to FIRINOX*

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### TEC16-Terapias Avanzadas: Diferenciación, Regeneración y Cáncer

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Pancreatic cancer is a disease with a poor prognosis due to its late diagnosis. Once diagnosed, most patients receive chemotherapy regimens as FOLFIRINOX (leucovorin, 5-fluorouracil (5-FU), irinotecan and oxaliplatin). Regrettably, resistance mechanisms are recurrent causes of treatment failure. A strategy to overcome these resistance phenotypes is to dissect them in genetically-defined model systems that take into account the heterogenous genetic landscape of these tumors. KRAS is an oncogene, which is mutated in more than 95% of patients with pancreatic ductal adenocarcinoma (PDAC). The main objective is to evaluate the mechanisms underlying acquired resistance to the combination of 3 cytostatic agents: 5-FU, SN-38 and Oxaliplatin in PDAC in the context of distinct KRAS mutations.

KPOs were previously generated by transfecting LSL-KrasMUT organoids (G12D, G12V and G12R) with a plasmid expressing Cre recombinase and a sgRNA targeting Tp53. We have chosen three prevalent mutations in PDAC as KRAS-G12D (40%), KRAS-G12V (32,5%) and KRAS-G12R (18%), to then, induced chemoresistance in the different KPOs by continuously treating them with increasing concentrations of FIRINOX regimen. To assess the acquisition of chemoresistance, cell viability was measured. Then, chemoresistant (chr) and non-chemoresistant KPOs were transplanted orthotopically into the tail of the pancreas of syngeneic mice (C57Bl/6N). Harvested tumors have been profiled by immunohistochemistry and flow cytometry at final time point.

We have already induced chemoresistance to FIRINOX in 3 KPO-KRASG12D, 3 KPO-KRASG12V and 3 KPO-KRASG12R lines. Preliminary data shows that in vitro chr-KPO lines tolerate a higher concentration of FIRINOX regimen. The initial characterization in vivo exhibits phenotypic changes among chr and non-chr-tumors expressing specific KRAS mutations. This preliminary data is the seed to continue our research strategy, that seeks to unravel the connection between specific-KRAS mutations, tumor heterogeneity and treatment resistance. In the near future, we hope to identify new vulnerable targets to design new therapy approaches depending on the specific KRAS mutation in the context of chemoresistance.



## *Acción de los exosomas derivados de células madre mesenquimales de médula ósea en la modulación de parámetros fibroblásticos asociados con la regeneración tisular*

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TEC17-Biotejsalud

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En el proceso de cicatrización los fibroblastos son esenciales, ya que sintetizan factores cruciales en la formación de la matriz extracelular. Las heridas crónicas son aquellas que presentan un proceso de inflamación prolongada, aumentando el tiempo de cicatrización y presentando un mayor riesgo de infección. Entre los abordajes novedosos en regeneración tisular, destacan terapias avanzadas como el uso de células madre mesenquimales (MSC), así como el uso de los exosomas liberados por las mismas como biomoléculas cruciales en la comunicación intercelular y la regeneración tisular. Por otro lado, compuestos fenólicos como oleocantal y oleuropeína, poseen propiedades que mejoran la regeneración tisular al estimular a los fibroblastos y promover la síntesis de colágeno.

Se obtuvieron exosomas derivados de células madre mesenquimales de médula ósea sin condicionar (ExoBMMSC), y condicionados con oleocantal (OCExoBMMSC) a dosis 25  $\mu$ M y oleuropeína (OPExoBMMSC) a dosis 100  $\mu$ M. La caracterización incluyó técnicas como microscopía electrónica, Western blot y mediciones de Zeta Potencial. Se realizaron ensayos de tratamiento con ExoBMMSC (dosis 1,2 y 3  $\mu$ g/mL), OCExoBMMSC (dosis 1,2 y 3  $\mu$ g/mL) y OPExoBMMSC (dosis 1,2 y 3  $\mu$ g/mL) sobre fibroblastos dérmicos humanos para evaluar la proliferación celular, la cicatrización in vitro mediante scratch, perfil genético de los genes COL-I, VEGF y TGF- $\beta$ , así como la medición de citoquinas y la proliferación en un medio inflamado. Los datos se analizaron estadísticamente con ANOVA.

Los resultados pusieron de manifiesto como los diferentes tratamientos mostraron una tendencia al alza en las técnicas de proliferación, migración, inhibición de la inflamación en medio inflamado in vitro, así como en la expresión de marcadores genéticos importantes en el proceso de reparación de tejidos. A destacar el efecto significativo a nivel estadístico de los tratamientos con OCExoBMMSC y OPExoBMMSC, siendo especialmente relevante en las dosis de 1 y 3  $\mu$ g/mL respectivamente.

Estos datos abren la puerta al uso de este tipo de terapia en el abordaje de lesiones tisulares de difícil cicatrización, aunque sigue siendo necesaria una mayor investigación en este sentido para determinar las vías de acción de estas nanopartículas, tanto condicionadas como sin condicionar.

## *Exosomas derivados de células madre mesenquimales de médula ósea promueven la proliferación y migración de osteoblastos humanos*

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**TEC17-Biotejsalud**

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La regeneración del tejido óseo se lleva a cabo fundamentalmente mediante regeneración celular y producción de matriz mineral. Sin embargo, determinadas patologías como la osteoporosis y circunstancias como el envejecimiento reducen esta capacidad. Esto ha puesto de manifiesto la necesidad de desarrollar nuevas estrategias para promover la cicatrización ósea. En este contexto, los exosomas derivados de células madre mesenquimales han emergido como agentes terapéuticos acelulares prometedores para la regeneración de tejidos, entre otros el tejido óseo. Estas vesículas de tamaño nanométrico han demostrado eficacia en la cicatrización de heridas, lesiones cerebrales, osteoartritis y enfermedades óseas, ofreciendo una nueva esperanza para mejorar la curación ósea.

Se obtuvieron exosomas derivados de células madre mesenquimales de médula ósea (ExoBMMSC). La caracterización incluyó técnicas como microscopía electrónica, Western blot y mediciones de Zeta Potencial. Se realizaron ensayos de tratamiento con ExoBMMSC (dosis 1,2 y 3 µg/mL) sobre osteoblastos primarios humanos (Hob) para evaluar la proliferación celular y la cicatrización in vitro mediante scratch. Los datos se analizaron estadísticamente con ANOVA.

Los resultados pusieron de manifiesto como los ExoBMMSCs en las diferentes dosis usadas (1, 2 y 3 µg/mL), promovieron la proliferación de Hob obtenidos de cultivos primarios a las 24 horas del tratamiento. Las dosis que resultaron significativas a nivel estadístico fueron la de 2 y 3 µg/mL. Los resultados obtenidos tras 48 y 72 horas no mostraron significación estadística. Por su parte, en el ensayo de cicatrización se puso de manifiesto como las diferentes dosis de ExoHUCMSCs fomentaron de forma significativa la cicatrización de los Hob a las 12 y a las 24 horas.

Los ExoBMMSCs ofrecen una alternativa no celular que podría revolucionar el tratamiento de lesiones óseas y mejorar la cicatrización, marcando un avance significativo en el abordaje de enfermedades que afectan la salud ósea.

## *Potencial de los extractos fenólicos de alperujo en la modulación de la inflamación y la proliferación celular*

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TEC17-Biotejsalud

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Las heridas crónicas representan un problema de salud y un reto para Enfermería. La fase inflamatoria inicial y el estímulo adecuado de la proliferación de queratinocitos son esenciales para restaurar la integridad epidérmica; sin embargo, una inflamación mantenida, con sobreexpresión de citoquinas como IL1B, retrasa la cicatrización, por lo que resulta clave modular su expresión. En este contexto, compuestos vegetales como los extractos fenólicos de alperujo (EFAs) surgen como coadyuvantes prometedores.

Objetivo: Analizar el efecto de los EFAs sobre la proliferación y la expresión de IL1B en queratinocitos estimulados con LPS.

Queratinocitos humanos (línea celular HaCaT) se cultivaron en presencia de LPS durante 24 h y, posteriormente, se trataron 24 h adicionales con diferentes concentraciones de EFAs. La proliferación se evaluó mediante la técnica espectrofotométrica del MTT y la expresión de IL1B en sobrenadantes mediante ELISA.

El LPS redujo la viabilidad celular frente al control, mientras que la co-incubación con distintas concentraciones de EFAs ( $10^{-2}$ ,  $10^{-3}$ ,  $10^{-6}$ ,  $10^{-7}$ ,  $10^{-8}$  y  $10^{-11}$  %) revirtió este efecto, aumentando significativamente la absorbancia hasta valores similares o incluso superiores al control, con un máximo alrededor de  $10^{-7}$ – $10^{-8}$  %, y sin evidencias de citotoxicidad en el rango estudiado. De forma paralela, los EFAs regularon la respuesta inflamatoria inducida por LPS reduciendo algunas de ellas su expresión.

Conclusiones: En conjunto, los resultados sugieren un efecto citoprotector y modulador de la inflamación dependiente de la dosis, con una posible ventana terapéutica de interés en cicatrización.

## *Análisis comparativo de la Interacción entre dos CARs anti-CD19 mediante herramientas bioinformáticas*

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TEC24-Grupo de Investigación en Hematología y Terapia Génica: HemaTerGe

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Los receptores de antígeno quimérico (CAR) representan una estrategia innovadora en inmunoterapia, ofreciendo alternativas efectivas para el tratamiento de diversos tipos de cáncer. Sin embargo, aún falta por mejorar la eficacia y seguridad, especialmente en tumores sólidos, donde la interacción antígeno-CAR es clave. En este estudio, se realizó un análisis bioinformático comparativo de la interacción entre dos CARs clínicos dirigidos contra la diana CD19, los cuales presentan una alta afinidad: Tisagenlecleucel (Kymriah®), un CAR patentado por Novartis y ARI-0001, un clon diferente diseñado en el Hospital Clínic de Barcelona.

Objetivo: establecer un protocolo bioinformático empleado para caracterizar la interacción entre los CARs y su diana mediante herramientas de acoplamiento molecular.

Para evaluar la interacción de estos CARs con CD19, se emplearon herramientas de acoplamiento molecular para modelar sus complejos proteína-proteína (AlphaFold2), junto con visualizadores estructurales (Pymol y ChimeraX) y metodologías para determinar los residuos clave en la interacción (Prodigy, PyDockEneRes y PDBePISA).

Se determinaron las diferencias estructurales entre ambos CARs y tanto la estabilidad como la afinidad de unión a su antígeno objetivo fueron evaluadas. El CAR Tisagenlecleucel-CD19 diseñado por Novartis presenta una afinidad predicha mayor que el CAR ARI0001-CD19. Por su parte, el CAR ARI0001-CD19 presenta un mayor número de aminoácidos interaccionantes señalados por al menos dos métodos.

## *Next-generation inducible promoters for safer and more specific CAR-T therapies*

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### TEC24-Grupo de Investigación en Hematología y Terapia Génica: HemaTerGe

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Chimeric antigen receptor (CAR) T cell therapy has revolutionized the treatment of refractory B-cell malignancies, achieving remarkable clinical outcomes. However, its efficacy in solid tumors remains limited, and severe toxicities such as cytokine release syndrome (CRS) pose major barriers to broader application. Precise regulation of transgene expression could improve both efficacy and safety by restricting the activity of therapeutic payloads to tumor-specific contexts. Activation-inducible promoters such as NFATsyn represents one such approach, but this promoter shows basal activity without induction and sustained activity independent of stimulation, which undermines their reliability

We designed chimeric promoters by combining transcription factor binding sites with promoters from genes involved in T cell activation (first-generation promoters). Promoter sequences were inserted into lentiviral vectors to drive GFP reporter expression, enabling quantitative assessment of promoter activity. Primary human T cells were transduced, and promoter activity was analysed after TCR activation. A second-generation promoter (named aiPROM2.1) was further developed by introducing a negative regulatory sequence designed to suppress transcription. Mutational analyses were performed to identify regulatory domains responsible for suppressive activity

In contrast to NFATsyn, which displayed persistent activity post-activation, the initial chimeric promoters exhibited similar behaviour but higher basal expression levels. The introduction of a negative regulatory sequence generated aiPROM2.1, which demonstrated markedly reduced basal activity. However, specific activation upon stimulation was attenuated compared to NFATsyn and original chimeric promoter. Deletion of the 3' terminal region of aiPROM2.1 restored promoter responsiveness, indicating the suppressor function localized to this domain. NFAT and aiPROM2.1 promoters are being tested in CAR-T cells, and activity was determined after different induction condition (cytokines, integrins and antigen).

We have developed an inducible promoter with similar behaviour than NFATsyn with higher specific and unspecific activity. The addition of a negative regulatory sequence blocks promoter activity in T cells activated through TCR. This promoter may have a potential CAR-specific effect.



## *ALHAMBRA: A Novel Promoter for Activation-Dependent Expression in Fourth-Generation CAR-T Cells*

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**TEC24-Grupo de Investigación en Hematología y Terapia Génica: HemaTerGe**

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The effectiveness of CAR-T therapy remains limited in solid tumors due to different factors. Fourth-generation CAR-T cells, engineered to secrete therapeutic molecules, offer a potential solution, but their secretion must be tightly regulated to avoid off-tumor toxicity. To achieve controlled secretion, several systems use activation-inducible promoters containing multiple NFAT transcription factor binding sites (TFBS), known as pNFATsyn and NFATmIL2 depending on their design. However, these promoters still show basal activation-independent expression, known as leakiness.

To reduce this leakiness, our objective is to design improved NFAT-based promoters that minimize basal expression while maintaining strong activation-induced expression, enabling safer gene regulation.

We engineered new NFAT-based promoters incorporating additional TFBS that may allow more precise control over the expression of genes of interest. We have named this platform ALHAMBRA.

ALHAMBRA promoters were delivered by lentiviral vectors (LVs) expressing eGFP as a reporter gene and used to transduce T cells and CAR-T cells (ARI - CARαCD19). eGFP expression was measured under different activation states by flow cytometry. Control cells were transduced with NFATmIL2-eGFP and NFATsyn-eGFP LVs. Transduced T and CAR-T cells were exposed to different stimuli, including anti-CD3/anti-CD28 antibodies, antigen positive cells (CD19+) and antigen-negative cells. The effect of removing individual TFBS on promoter activity was also evaluated to determine the role of each TFBS.

ALHAMBRA-based LVs exhibited lower basal gene expression in non-activated T cells and CAR-T cells compared with LVs containing NFATmIL2 or NFATsyn promoters. This reduction can be observed both when comparing unstimulated T cells and when comparing CAR-T cells cocultured with antigen-negative (CD19-) target cells, while expression is strongly induced upon T-cell activation or when CAR-T cells are cocultured with CD19+ tumor cells. Additionally, deletion of individual TFBS results in an increase in basal eGFP expression, indicating that these TFBS contribute to repressing transgene expression in non-activated cells.

In conclusion, the ALHAMBRA platform provides a tightly regulated promoter system that minimizes basal gene expression and supports safer, activation-dependent control of genes of interest in fourth-generation CAR-T cells.

## *Exploring Dual-Guide RNA architectures to enhance CRISPR/Cas13-based diagnostics*

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**TECe18-Nanochembio**

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CRISPR-Cas systems have transformed molecular biology by enabling programmable nucleic acid recognition and manipulation. Cas13, an RNA-guided RNase, has attracted particular attention for its ability to target RNA with high sensitivity, offering unique opportunities in both diagnostics and therapeutics. However, its remarkable reactivity often compromises target discrimination, as even single-nucleotide mismatches may not fully prevent activation. This limitation poses a major challenge for applications requiring strict specificity, such as the detection of point mutations or viral variants.

To overcome this, we developed a dual-guide RNA architecture for Cas13 that integrates two RNA components (dcrRNA and dtracrRNA) connected through short nucleotide linkers (patent EP4414452A1).

Dual-guide RNAs were designed by linking dcrRNA and dtracrRNA domains through 5- and 7-nucleotide RNA spacers. Structural modeling was performed using AlphaFold 3 to predict RNA-protein interactions and assess conformational stability relative to the conventional single-guide Cas13 complex. The interaction between Cas13 and dual-guide complexes was examined by electrophoretic mobility shift assays (EMSA). Cleavage activity was assessed through in vitro RNase assays, testing both cis and trans cleavage using a fluorescent reporter. Specificity was evaluated against diverse RNA targets containing single-nucleotide variants (SNVs) corresponding to KRAS and SARS-CoV-2 genomic sequences. For translational assessment, Cas13 and dual-guide RNAs were delivered into cell models via nucleofection.

Dual-guide Cas13 complexes efficiently bound and cleaved target RNA, maintaining robust cis- and trans-RNase activity comparable to the single-guide system. EMSA confirmed stable RNA-protein assembly, while AlphaFold 3 modeling showed conserved structural architecture, validating proper scaffold folding. Notably, the dual-guide system improved mismatch discrimination, enabling precise detection of single-nucleotide variants such as KRAS-G12D and KRAS-G12C over the wild-type allele, and accurate identification of SARS-CoV-2 RNA variations. Cellular assays further supported the system's potential activity in biologically relevant contexts. Altogether, this dual-guide design expands Cas13 versatility by introducing highly-specific target recognition without compromising efficiency. By enhancing mismatch recognition while retaining strong catalytic performance, it establishes a robust foundation for next-generation RNA-targeting technologies in diagnostics and precision medicine.

## *Development of Hybrid Nanoparticles for Dual Activation Cancer Treatment via Magnetic and NIR Fields*

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**TECe18-Nanochembio**

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Current cancer therapies often lack the capability to selectively target tumor tissues without demaging healthy cells. Additionally, many drug delivery systems exhibit poor control over release kinetics and biodistribution. Furthermore, monotherapy approaches frequently fail to address the heterogeneity and adaptability of tumor microenvironments. Therefore, there is a critical need for integrative solutions that combine diagnostic and therapeutic functions within a single, controllable system.

Our project, CoCoGel, aims to develop a nanotechnology-based platform to overcome current cancer therapy challenges. Our first approach is focused on the synthesis of hybrid nanoparticles composed of gold (Au) anchored on magnetic iron oxide ( $\text{Fe}_3\text{O}_4$ ) cores, encapsulated within a silica ( $\text{SiO}_2$ ) shell. Gold offers excellent biocompatibility and strong optical properties (plasmonic resonance), useful for photothermal therapies. Magnetite ( $\text{Fe}_3\text{O}_4$ ) is superparamagnetic at the nanoscale, allowing magnetic guidance and heat generation under alternating magnetic fields (magnetic hyperthermia). The silica shell ensures nanoparticle stability and provides a versatile surface for functionalization with tumor-targeting ligands. A second approach utilizes gold nanorods (AuNRs) coated with a silica ( $\text{SiO}_2$ ) shell and with magnetite ( $\text{Fe}_3\text{O}_4$ ) nanoparticles functionalized on the external surface.

Currently, we have developed several hybrid nanosystems that have been thoroughly characterized. They exhibit good biocompatibility and can be effectively activated using magnetic fields and near-infrared (NIR) radiation.

By integrating multiple therapeutic modalities into a single platform, this work establishes a basis for the development of next-generation smart biomaterials for localized, effective, and minimally invasive cancer treatment. This integrative system holds promise for improved precision and efficacy in cancer treatment.

## *Nanocatalytic Platforms and Bioorthogonal Prodrug Systems to Enhance CAR-T Cell Immunotherapy in Breast Cancer*

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**TECe18-Nanochembio**

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Chimeric antigen receptor (CAR)-T cell therapy has revolutionized cancer treatment by harnessing patients' own immune cells to recognize and eliminate tumor-associated antigens through localized cytotoxic responses. Although highly effective against B-cell malignancies, its efficacy against solid tumors—such as breast cancer—is limited by challenges including antigen loss, an immunosuppressive tumor microenvironment and T-cell exhaustion. To overcome these hurdles, innovative strategies are urgently needed to enhance the antitumor activity of CAR-T cells directly at the tumor site. Our project integrates nanotechnology, bioorthogonal catalysis, and chemical biology to engineer precise biomedical solutions.

Key objectives include designing and characterizing functionalized metal-based nanosystems for robust binding to CAR-T cells. The characterization and confirmation of this nano-decoration process were performed using flow cytometry and confocal microscopy imaging. Concurrently, a library of prodrugs designed for rapid activation via nanosystem-mediated bioorthogonal catalysis was developed.

We have successfully synthesized first-generation nanosystems and demonstrated their stable and selective surface binding to CAR-T cells without impairing viability. Confocal imaging and flow cytometry confirm efficient immune cell decoration, while preliminary prodrug activation assays validate the catalytic activity of the platform. This work introduces a novel approach to augment CAR-T cell immunotherapy by imparting catalytic functions, enabling in situ drug activation. This strategy offers a promising means to overcome therapeutic resistance in solid tumors, advancing targeted drug delivery towards clinical translation and establishing a new paradigm for CAR-T-based smart nanomedicines.

## *Fluorescent Copper Nanocatalyst for Tracking Azide-Alkyne Cycloadditions in both solutions and cells*

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### TECe18-Nanochembio

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Since the introduction of click chemistry by Sharpless and Meldal and its expansion to living systems through Bertozzi's bioorthogonal chemistry, these reactions have transformed chemical biology. Among them, the copper-catalyzed azide-alkyne cycloaddition (CuAAC) is the most prominent example, recognized with the 2022 Nobel Prize in Chemistry for its efficiency and selectivity in forming stable triazoles.

Despite its potential, the biological use of CuAAC is hindered by copper toxicity, oxidation state instability, and reactive oxygen species (ROS) formation, which limit its biocompatibility. Although copper-free click reactions exist, many alkyne-containing precursors would still benefit from CuAAC chemistry if non-toxic copper systems were available.

Here, we report the design and synthesis of a tracker nanocatalyst, Cu@BTAA-Cy5-NPs, composed of copper metallofluorescent nanoparticles stabilized by the BTAA ligand and functionalized with the fluorescent dye Cy5. These monodisperse nanoparticles display robust CuAAC catalytic activity and intrinsic fluorescence, enabling real-time monitoring of nanoparticle localization and catalytic progress within living cells.

A comprehensive study revealed that Cu@BTAA-Cy5-NPs exhibit high catalytic efficiency under mild conditions, excellent recyclability, and broad substrate scope with high yields and short reaction times. Moreover, these nanocatalysts demonstrate outstanding biocompatibility, efficient cellular uptake, and negligible toxicity, representing a new generation of non-biotoxic heterogeneous copper catalysts that combine superior catalytic performance with fluorescence tracking for intracellular and bioorthogonal CuAAC applications.



## *A Pd-modified porphyrinic MOF for combined bioorthogonal catalysis and photodynamic therapy*

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TECe18-Nanochembio

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Standard chemotherapeutics have several limitations, including systemic side effects, poor bioavailability, and short half-lives. Therefore, the development of improved anticancer strategies is mandatory. One promising approach involves the use of abiotic transition-metal catalysts (e.g. Pd) to convert inactive prodrugs into cytotoxic drugs at the tumour site. In parallel, light-triggered therapies such as photodynamic therapy (PDT) offer spatial and temporal control by generating ROS through photosensitizers, with porphyrin-based structures among the most studied. Our aim is to develop a Pd-functionalized porphyrin-based metal-organic framework (MOF) that combines the in situ depropargylation of an inactive derivative of 5-fluorouracil (Pro-5FU) and PDT under visible light irradiation.

Two architectures were synthesised using PCN-222 as framework: Pd@PCN, featuring Pd(II) coordinated to the porphyrinic rings, and PCN/Pd, bearing Pd(0) nanoparticles on the MOF surface. Activation experiments were carried out under physiological conditions using Pro-Resorufin as a Pd-sensitive probe. Cellular assays were performed in BxPC3 cells, with viability measured after 5 days of treatment using PrestoBlue® reagent.

Pd@PCN showed the highest catalytic activity with an 80% conversion of the off-on sensor at 24 h and demonstrated minimal toxicity even at the highest Pd concentration tested (30 µg/mL). Pd@PCN efficiently activated Pro-5FU, as confirmed by HPLC, with complete conversion to 5FU. Additionally, cells incubated with Pd@PCN and irradiated exhibited time-dependent phototoxicity. A combined chemo-PDT experiment revealed that co-treatment with Pd@PCN and Pro-5FU under irradiation produced greater inhibition of cell proliferation than individual treatments, establishing this system as a promising dual-activity nanoplatform for cancer treatment.

## *Beyond Standard Treatment: Can Exercise and Mindfulness Enhance Disability in Individuals with Chronic Primary Low Back Pain? The BACKFIT project*

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### TECe20-Rehabilita-T: Avances e innovación en rehabilitación y promoción de la salud

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Individuals with chronic primary low back pain (CPLBP) usually present intense pain which is often accompanied by disability. The aim of this study was to compare the effects of exercise and a multimodal approach (exercise+mindfulness) with a usual rehabilitation care group on disability due to pain in individuals with CPLBP.

Of 105 randomized participants, 53 individuals with CPLBP aged  $52 \pm 9$  years attended >65% of the program and were analyzed: control group (CG, n=19), exercise group (EG, n=14) and exercise+mindfulness group (EMG, n=20). All groups completed an 8-week intervention twice weekly (45 minutes/session). The CG received usual care (stretching and motor control exercises). The EG and EMG performed supervised muscle-strengthening and motor control exercise. Additionally, the EMG attended weekly mindfulness session (2.5 hours/session). Disability due to pain was assessed with the Oswestry Disability Index. Assessments were performed pre- and post- intervention and after a 2-month detraining period (re).

Repeated measures (Wilcoxon test) showed that EG (median (Me)=-12.00; Interquartile range (Q1-Q3)= -16.44, -5.00;  $p=0.003$ ) and EMG (Me= -3.11; Q1-Q3= -12.00, 0.00;  $p=0.005$ ) reduced disability due to pain after the intervention. CG did not show improvements (Me= -8.00; Q1-Q3= -11.11, -2.00;  $p>0.05$ ). After the detraining period, no significant intra-group differences were found compared to the baseline (all,  $p>0.05$ ). Kruskal-Wallis test showed no significant between-group differences in post-intervention and follow-up changes from baseline for any of the outcomes (all,  $p>0.05$ ). To conclude, both exercise alone and exercise and mindfulness approach were effective compared to usual rehabilitation care in reducing disability due to pain in individuals with CPLBP. Future research is warranted to explore whether multimodal interventions incorporating higher exercise intensity, yield superior effects in this population.

## *Melatonin Ameliorates Heart Failure by Improving Ventricular Remodeling and Ejection Fraction in Male ZDF Rats*

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### MP-19 Nutrición, Metabolismo, Crecimiento y Desarrollo

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Heart failure (HF) is a clinical syndrome in which the heart is unable to pump sufficient blood to meet the metabolic demands of the body. The prevalence of HF is higher in men, and numerous studies have reported marked sex-related differences in both cardiac structure and function. Melatonin is a hormone that regulates circadian rhythms and exhibits pleiotropic cardioprotective properties. This molecule mitigates cardiac injury and physiological disturbances through its potent antioxidant, anti-inflammatory, and anti-apoptotic effects. In addition, several studies using muscle samples have demonstrated that melatonin modulates mitochondrial dynamics, thereby contributing to the restoration of mitochondrial function and energy production.

In this study, 9-week-old male obese diabetic Zucker rats (ZDF) and their lean littermates (ZL) were divided into four subgroups (n=4): control (C), melatonin-treated (M), dapagliflozin-treated (D), and dapagliflozin and melatonin-treated (DM). Treatments were administered orally at a dose of 10 mg/kg/day for 17 weeks. Cardiac function was assessed by echocardiography and plethysmography. Western blot analysis was performed on the left ventricle (LV) to study mitochondrial dynamics and calcium metabolism. Histological stains (H&E, Masson's trichrome, picrosirius red, and oil red O) were used to analyze remodeling, fibrosis, inflammation, hypertrophy, and lipid accumulation.

Echocardiography revealed increased ejection fraction and ventricular volumes in M, D, and DM ZDF rats, while plethysmography showed reduced systolic and diastolic pressures across all treatments. Histological analyses indicated that melatonin and dapagliflozin decreased myocardial fibrosis, lipid accumulation, and cardiomyocyte hypertrophy, obtaining synergistic effects in DM ZDF rats. Cardiac metabolism and mitochondrial function were also improved in all groups, with enhanced outcomes in DM ZDF rats. Treatments also reduced mitochondrial fission and promoted fusion, with M ZDF rats yielding greater effects in fusion promotion. The combined treatment further reduced mitochondrial fission and enhanced fusion.

In conclusion, melatonin plays an important cardioprotective role by reducing ventricular remodeling and mitochondrial dysfunction, and its combination with dapagliflozin may represent a novel and more effective therapeutic strategy for the management of heart failure.

## *Conexiones genéticas entre la depresión mayor y la obesidad: un enfoque desde la secuenciación de nueva generación*

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### E05-Psiquiatría Bioambiental

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La depresión mayor (DM) y la obesidad son trastornos complejos, altamente prevalentes y con un impacto significativo en la salud pública. Diversas evidencias epidemiológicas y genéticas respaldan una relación bidireccional entre ambas condiciones, probablemente mediada por mecanismos compartidos de disfunción neuroendocrina, inflamación crónica y vulnerabilidad genética. Sin embargo, los correlatos genéticos específicos de este fenotipo comórbido permanecen poco caracterizados. La secuenciación de exoma completo (WES) constituye un enfoque de nueva generación ideal para identificar variantes genéticas comunes, raras o novedosas asociadas a la DM, la obesidad y su fenotipo comórbido. En este proyecto se realizará un análisis WES en una submuestra de 654 individuos del estudio PISMA-ep.

la submuestra PISMA-ep de 654 individuos se divide en cuatro grupos: i) individuos con DM sin obesidad; ii) con obesidad sin DM; iii) con fenotipo comórbido; iv) controles sanos. Los datos de secuenciación se procesarán con un pipeline estandarizado que incluirá control de calidad, alineamiento y refinamiento, anotación funcional y filtrado de variantes raras y posteriormente se optimizará. Se realizarán análisis de rutas biológicas para ver los procesos implicados en las variantes identificadas y análisis funcionales a nivel de variante y gen. Posteriormente se validarán los resultados con bases de datos públicas y validación experimental.

Se espera que la identificación y caracterización de perfiles genéticos de susceptibilidad contribuya a reconocer a la población en riesgo y desarrollar estrategias de prevención dirigidas y personalizadas, lo que no solo mejorará la calidad de vida de los pacientes y reducirá comorbilidades, sino que también optimizará la calidad asistencial y generará un impacto positivo en la eficiencia del Sistema de Salud. Se prevé identificar rutas metabólicas y de señalización no descritas previamente en relación con la enfermedad. Este hallazgo podría ampliar el conocimiento sobre los mecanismos fisiopatológicos subyacentes y revelar nuevos puntos de intervención terapéutica, abriendo la posibilidad de reposicionar fármacos ya existentes o diseñar terapias dirigidas. Asimismo, se plantea el desarrollo de un pipeline computacional reproducible que integre las principales fases del análisis de datos ómicos, facilitando su reutilización por otros grupos.

## *Dissecting the Molecular Basis of Epigenetic Memory by the H3K9 Methylation System*

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### **Ae21-Epigenética en Envejecimiento y Cáncer**

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Loss of cell identity caused by epigenetic instability is emerging as a fundamental hallmark of ageing and disease. As therapeutic manipulation of the epigenome becomes a frontier in modern medicine, understanding how cells preserve transcriptional memory is essential, yet the molecular basis of this process remains elusive. Methylation of histone H3 at lysine 9 constitutes a key repressive chromatin mark associated with heterochromatin formation and transcriptional silencing of genes and repetitive elements. The faithful transmission of these repressive states through cell division requires epigenetic memory mechanisms that ensure the inheritance and maintenance of specific expression and silencing programs across cell generations.

In this work, we examined multiple components of the H3K9 methylation machinery using diverse molecular and cellular approaches to dissect their contributions to the maintenance and inheritance of epigenetic repression.

Our findings provide new insights into how H3K9-dependent mechanisms safeguard transcriptional states during cell division, potentially informing future strategies to counteract epigenetic dysfunctions underlying ageing and disease.



*Modulating the epigenome of cancer cells as an innovative therapeutic approach*

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**Ae21-Epigenética en Envejecimiento y Cáncer**

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Current cancer treatments primarily aim to remove malignant cells or induce their cytotoxicity through surgery, radio-, chemo-, or immunotherapy. However, these interventions often cause substantial toxicity in healthy tissues and incomplete therapeutic responses, leading to patient distress and economic burden on public health system.

Epigenetic factors have emerged as key druggable targets in cancer and small-molecule inhibitors of epigenetic regulators (epidrugs) are being developed or approved for clinical use. Nonetheless, their clinical success has so far been limited, likely in part due to ineffective therapeutic design. Hence, innovative approaches are required to fully harness the potential of epigenetic therapy in cancer. Here, we provide new insights toward achieving this goal.

By combining transcriptomics, epigenomics, cancer cell lines, and mouse xenograft models of non-small cell lung cancer, melanoma, multiple myeloma, and acute myeloid leukaemia, we aim to analyse the downstream effect of treating cancer cells with small-molecule clinically approved epidrugs on their epigenome, transcriptome, and tumorigenic capacity.

We exhibit the results of new possible experimental designs with epidrugs to treat cancer cells and fully take advantage of the potential of those small-molecule inhibitors, which could even be combined with current therapies.

*Rewiring the epigenome to change cancer chemosensitivity*

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**Ae21-Epigenética en Envejecimiento y Cáncer**

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Cancers represent the second leading cause of mortality occurring worldwide, accounting for up to 10 million deaths in 2020. This situation has driven the development of numerous therapeutic strategies that, while initially effective, may lose efficacy over time due to the emergence of resistance, progressively narrowing the range of drugs available for the treatment of a given tumor. Emerging evidence reveals epigenetics plays a significant role in cancer behavior, controlling processes such as chemoresistance or even acting as a major driver of disease. Therefore, and although epigenetic therapy was first designed seeking cytotoxic activity, we believe it has the potential, through changes in the epigenome, to affect how cancer respond to other cytotoxic drugs and synergize with them.

Using epigenomic analysis and mouse xenograft assays we aim to study the impact of the epigenome on drug response. Our results suggest that treatment with epigenetic drugs may induce changes in how cancer cells respond to different chemotherapeutic agents.

## *Relación genética entre la artritis idiopática juvenil y el hipotiroidismo*

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### E05-Psiquiatría Bioambiental

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La artritis idiopática juvenil (AIJ) es una enfermedad reumática crónica que causa inflamación persistente de las articulaciones en niños menores de 16 años. Se ha observado que los pacientes con AIJ presentan una prevalencia de hipotiroidismo aproximadamente seis veces mayor que la población general. Esta comorbilidad podría ser debida a mecanismos genéticos. El propósito fundamental de este trabajo fue estudiar si la comorbilidad observada mediante estudios epidemiológicos entre la artritis idiopática juvenil (AIJ) y el hipotiroidismo puede deberse, al menos en parte, a una relación de causalidad genética.

Se llevaron a cabo dos aproximaciones. En primer lugar, se realizó un análisis preliminar de randomización mendeliana (MR), con datos obtenidos a partir de estudios de asociación de genoma completo (GWAS), de ambas enfermedades, utilizando mutaciones de tipo SNP como variables instrumentales. Por otro lado, para la segunda aproximación, se seleccionaron exclusivamente los SNPs que habían sido firmemente asociadas a AIJ en estudios previos. Además, se llevó a cabo una investigación sobre la pleiotropía, manifiesta cuando una variable instrumental afecta al hipotiroidismo pero no a través de la AIJ, sino por rutas biológicas alternativas.

Los resultados mostraron que la genética de la AIJ tiene un efecto significativo sobre el riesgo de padecer hipotiroidismo en, al menos, uno de los métodos utilizados, estos resultados permanecieron después de los análisis de sensibilidad. Asimismo, se detectaron SNPs pleiotrópicos presentes en genes asociados con la función del sistema inmunológico, como STAT4, SH2B3 y FAS, pudiendo estos estar implicados en la comorbilidad observada entre las dos enfermedades.

Este análisis muestra una relación de causalidad genética entre la AIJ y el hipotiroidismo, además de presentar un conjunto de SNPs pleiotrópicos potencialmente implicados en su comorbilidad, encontrados en genes vinculados con la función inmunitaria. Sin embargo, es necesario validar los resultados en cohortes más grandes, para aumentar la potencia estadística de este y posteriores estudios que permitan poder corroborar estos hallazgos y lograr su traducción clínica.

## *Simulation of protein adsorption on the surface of a graphene field-effect transistor (GFET) sensor*

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**TEC04-Nanoelectrónica**

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Graphene field-effect transistors (GFETs) have emerged as one of the most promising platforms for biosensing applications due to their exceptional electronic properties and high sensitivity to surface charge variations. Specifically, when antibodies are adsorbed onto the graphene surface, their charge distribution and orientation play a crucial role in determining the sensitivity and reproducibility of the sensor. In this study, we investigated the adsorption and orientation of antibodies on the surface of a biosensor using different parameters (surface charge density, ionic force...) through simulation.

A biosensor is an analytical tool that translates the effects of a biological recognition event into a measurable physical signal. The electrical characterization of a GFET is performed by measuring the drain current  $I_D$  as a function of the gate voltage  $V_{FG}$ . The functionalization of the biosensor will be carried out by adsorbing antibodies onto the surface. The initial conditions of the simulation define the initial state of the system before starting the simulation, determining the initial distribution and orientation of the antibodies.

The results obtained from Monte Carlo simulations using the Metropolis algorithm are presented. We study the distribution and orientation of antibodies on an unfunctionalized graphene surface.

1. On neutral surfaces and without functionalization at low ionic strength, adsorption is high with flat-on orientation dominating.
2. With positive density on the non-functionalized surface, the percentage of adsorption decreases, but end-on orientation is favored.
3. Negative charge gives rise to head-on orientations.
4. GFET with a functionalized surface favors end-on antibodies in all cases and, as in previous cases, surface coverage decreases as ionic strength increases.

## *Heterochromatin as the guardian of youthful epigenetic memory*

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### Ae21-Epigenética en Envejecimiento y Cáncer

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Age-related diseases, taken together, represent the leading causes of human suffering and mortality worldwide. However, the molecular basis of aging are still poorly understood. The discovery of a major molecular modulator underlying all aging processes would represent a major breakthrough in the development of holistic therapeutic interventions to alleviate multiple age-related diseases. Given the emerging literature on the causal role of epigenetics in aging, we aim to study whether loss of heterochromatin can directly accelerate the aging process. We expect such relation can be exploited in the future for generating better interventions to slow down age-related dysfunction.

Using murine models, we will study the impact of different genetic mutations and pharmacological interventions related to chromatin structure to the aging process.

As the project is still in its infancy, we present its latest developments as we set up the next experiment



## *Analysis of skin differentiation markers in severely burnt patients grafted with the UGRSKIN bioartificial skin substitute generated by tissue engineering*

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### TEC-03 Ingeniería Tisular

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**Background:** Treatment of severely burnt patients is challenging, and the main goal is to reestablish the epithelial barrier of the patient as soon as possible. However, patients with more than 50% of the body surface affected by burns require the use of bioartificial models of human skin developed in the laboratory. In this regard, we have previously generated the UGRSKIN bioartificial skin based on nanostructured fibrin-agarose biomaterials with dermal and epidermal cells cultured within and on the surface of this biomaterial, which was authorized by the Spanish Medicines Agency for consolidated use in the Spanish Health System (ref. 89618) (1). The objective of this work is to evaluate the histological and molecular mechanisms associated with the biointegration process of UGRSKIN in burnt patients.

**Methods:** UGRSKIN substitutes were generated from a skin biopsy obtained from healthy areas of the patient. In brief, biopsies were enzymatically digested, and stromal fibroblasts and epidermal keratinocytes were isolated and expanded in culture and combined with fibrin-agarose biomaterials. The final product was then nanostructured using a bioreactor. Patients from whom the biopsies were taken were treated with the artificial skin, and biopsies of the treated area were obtained after 1, 2 and 3 months of follow-up. Histological analyses were conducted at each time to determine the sequential expression of relevant skin markers and components.

**Results and conclusions:** Histological analysis of the biopsies revealed that UGRSKIN grafted in patients tended to show high levels of expression of intercellular junction proteins, such as PKG and claudin, and the epidermal differentiation markers involucrin and filaggrin, from the first month of the implant. However, the number of melanocytes and dendritic cells tended to increase with time, with low number of cells at month 1, and a high number of cells after 3 months of the graft. Regarding blood vessels, our study showed a high number of capillaries and arterioles from the first month, with higher number than control human skin in the superficial dermis. These results confirm the rapid biointegration process of the UGRSKIN substitute and support the clinical use of this medicinal product in severely burnt patients.

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## *Optimization of epithelial cell cultures by using a mixture of bioactive components*

Ortiz-Arrabal O1,2, Ávila-Fernández P1,2, González-Gallardo C2,3, Sánchez-Porras D1,2, García-García OD1,2, Martín-Piedra MA1,2, Chato-Astrain J1,2, Garzón I1,2, Carriel V1,2, Mesa-García MD2,4,5, Gómez-Llorente C2,4,5, Alaminos M1,2

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**TEC-03 Ingeniería Tisular**

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**Background:** Generation of human artificial tissues by tissue engineering is highly dependent on the availability of cell cultures that are normally established from human biopsies (1). Although certain cell types, such as the mesenchymal stem cells, are easy to keep and expand in culture, epithelial cells tend to show limited proliferation potential, which may hinder the generation of a bioengineered substitute of the human skin, oral mucosa or cornea. In the present work, we have evaluated several bioactive compounds as potential inducers of cell proliferation able to improve the current methods used in skin, oral mucosa and cornea tissue engineering.

**Methods:** Four bioactive components potentially useful in tissue engineering were evaluated on human epithelial cells, including maslinic acid (MA), phenolic extract (PE), DL-3,4-dihydroxyphenyl glycol (DHFG), and oleuropein (OLP). Cells were cultured for 24, 48 and 72h in the presence of increasing concentrations of each component, and cell viability and proliferation were analyzed at each time.

**Results and conclusions:** First, we found that all components were safe for the cells at the lowest concentrations, with cells showing very high viability using the LIVE/DEAD method and low DNA release to the culture medium. However, viability was significantly reduced at the highest concentrations, especially when the concentration of 80µg/mL was used. Regarding cell proliferation, we found that the use of MA, PE and OLP was able to significantly increase the number of cells, especially at the concentrations of 5-10µg/mL, with an increase in the metabolic activity analyzed by WST-1. These results support the use of the bioactive compounds analyzed in the present work to improve the generation of epithelial cell cultures for use in tissue engineering of the human skin, oral mucosa and cornea.

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## *In vivo evaluation of a multilayered acellular urethral substitute in laboratory rabbits*

Sánchez-Porras D1,2, Campos F1,2, Etayo-Escanilla M1,2, García-García OD1,2, Ortiz-Arrabal O1,2, Carriel V1,2, García JM1,2, Campos A1,2, Sánchez-Quevedo MC1,2, Fernández-Valadés R1,2

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**TEC-03 Ingeniería Tisular**

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**Background:** The human urethra has very limited regeneration potential, and treatment of structural defects of this organ is very complex. In this context, tissue engineering offers the possibility to treat severe conditions by using bioartificial tissues generated in the laboratory using different kinds of biocompatible biomaterials. Recently, we developed a biological urethral substitute by tissue engineering that demonstrated to be biomimetic to the human urethra (1). In the present work, we evaluated the biosafety of the acellular urethral substitute grafted on the urethra of laboratory rabbits.

**Methods:** A multilayered substitute of the urethra was generated in the laboratory by combining several layers of bioartificial tissue based on fibrin and fibrin-agarose biomaterials using nanostructuration methods. By applying control pressure and dehydration, the different layers of biomaterials can be fused to form a single structure consisting of several layers of biomaterials, as previously described (2). This multilayered urethra substitute was implanted at the ventral side of the rabbit urethra, after a defect had been created surgically on the ventral side of the native urethra, and animals were evaluated after 1 month of follow-up, and the implant site was evaluated for safety.

**Results and conclusion:** Preliminary results confirm that the urethral implant of a multilayered structure resembling the different layers of the urethra in laboratory rabbits is safe, and no side effects were found at the implant site or at distal organs. The macroscopical aspect of the implant was compatible with a tissue that was properly integrated and probably contributed to regeneration of the damaged urethra, without any signs of complications. Although future studies should be carried out, these preliminary work supports the biocompatibility and potential usefulness of a multilayered urethra generated by tissue engineering for the regeneration of the damaged urethra in laboratory rabbits.

**Acknowledgements:** This work was supported by Instituto de Salud Carlos III, Spanish Ministry of Science, Innovation and Universities, Grant FIS PI22/00059, and co-funded by the European Union.

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*Prevascularized fibrin-agarose skin substitutes with mesenchymal stem cells for enhanced biointegration of skin models*

Chato-Astrain J1,2, Ávila-Fernández P1,2, Sánchez-Porras D1,2, García-García OD1,2, Ortiz-Arrabal O1,2, Bermejo-Casares F1, Crespo PV1,2, Alaminos M1,2, Garzón I1,2, Martín-Piedra MA1,2

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**TEC-03 Ingeniería Tisular**

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**Background:** Rapid and stable vascularization is critical for the success of bioartificial skin substitutes in severely burned patients, as it ensures nutrient supply, oxygenation, and integration with host tissue (1). UGRSKIN, based on fibrin-agarose hydrogels, has demonstrated clinical efficacy in restoring the epithelial barrier (2), but its biointegration could be further improved by incorporating prevascularized cellular components. Mesenchymal stem cells (MSC) are known to promote angiogenesis, modulate inflammation, and support extracellular matrix remodeling. Combining MSC with endothelial cells within the scaffold under pro-angiogenic conditions may accelerate neovascularization and enhance graft survival.

**Methods:** Fibrin-agarose scaffolds were fabricated and combined with endothelial cells and MSC (bone marrow and adipose-derived), either undifferentiated or pre-differentiated toward vascular phenotypes. Constructs were cultured using pro-angiogenic conditions and induction media before implantation. Biointegration was assessed by histology and immunohistochemistry at 7 days post-grafting in experimental models.

**Results and Conclusions:** Prevascularized fibrin-agarose skin substitutes showed accelerated neovascularization and improved integration compared to standard UGRSKIN. MSC promoted the formation of branched vascular networks and enhanced extracellular matrix deposition. These findings suggest that incorporating MSC into fibrin-agarose scaffolds represents a promising strategy to develop next-generation skin substitutes with superior regenerative potential for burn patients.

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## *Signaling pathways orchestrating ex vivo maturation of human bioengineered dermo-epidermal skin substitutes*

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**Background:** Previous reports demonstrated that the fibrin-agarose bioartificial skin substitute UGRSKIN may be clinically useful for the treatment of severely burnt patients (1). However, the molecular mechanisms governing ex vivo maturation of this skin substitute remain poorly understood. Determining signaling dynamics during culture is essential to guide strategies enhancing biomimicry and predicting graft performance.

**Methods:** Two human skin substitutes were analyzed: an epithelial substitute (ESS) and a stromal-epithelial substitute containing fibroblasts (SESS). Constructs were cultured under air-liquid conditions for 14 days. Protein arrays were used for profiling key functional (proliferation, epidermal/dermal differentiation, stress/apoptosis, inflammation) and signaling pathways (MAPK, AKT, JAK/STAT, NF- $\kappa$ B, TGF- $\beta$ ).

**Results and Discussion:** SESS promoted a more balanced and physiological maturation compared to ESS models. Stromal-epithelial constructs activated MAPK and AKT pathways (ERK1/2, RAF-1, PDK1, GSK3 $\beta$ ), supporting proliferation and tissue organization, while late TGF- $\beta$  signaling (SMAD1, c-Jun) enhanced dermal remodeling and epidermal differentiation. Furthermore, SESS attenuated excessive inflammatory and stress responses linked to JAK/STAT (STAT3) and NF- $\kappa$ B, creating a signaling environment favorable for structural stability and functional maturation. This coordinated signaling environment in stromal-epithelial models suggests that fibroblast-derived cues are essential for guiding epidermal differentiation and structural stability.

**Conclusions:** Mapping signaling pathways during ex vivo development provides actionable criteria for optimizing UGRSKIN manufacturing which could improve predictability and clinical outcomes of human bioengineered skin for use in burnt patients.

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*Ex vivo extracellular matrix maturation of UGRSKIN fibrin-agarose skin substitutes resembles the development process of the native skin*

Ávila-Fernández P1,2, Campos F1,2, García-García OD1,2, Ortiz-Arrabal O1,2, Sánchez-Porras D1,2, Etayo-Escanilla M1,2, Bermejo-Casares F1, España-López A3, Alaminos M1,2, Chato-Astrain J1,2

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**TEC-03 Ingeniería Tisular**

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**Background:** The extracellular matrix (ECM) plays a fundamental role in supporting skin structure and function, influencing cell adhesion, signaling, and tissue regeneration (1). Several models of human bioartificial skin substitutes have been developed, including UGRSKIN, based on fibrin-agarose hydrogels, which proved to be safe and clinically useful for the treatment of burnt patients. However, understanding the complex process of ECM maturation that takes place during ex vivo development of this artificial tissue is essential to optimize graft quality and integration. In the present work, we carried out an ex vivo analysis of the ECM at different time points to characterize the development process of UGRSKIN.

**Methods:** UGRSKIN substitutes were generated using a cellular stromal layer based on fibrin-agarose biomaterials with fibroblasts within and a stratified epithelium on top. This artificial tissue was cultured under controlled conditions and sequentially analyzed at different time points. ECM maturation was analyzed by immunohistochemistry and a molecular profiling of key components, including collagens (I, IV, VII), proteoglycans (decorin, agrin, perlecan), and basement membrane proteins (fibronectin, nidogen) was carried out. Activation of signaling pathways such as TGF- $\beta$  was also assessed.

**Results and Conclusions:** UGRSKIN dermo-epidermal skin models exhibited a progressive and organized deposition of ECM molecules resembling the native dermis, with early basement membrane formation and increased expression of structural proteins over time. These findings confirm that epithelial-stromal interaction is critical for ECM development and suggest that ex vivo maturation of UGRSKIN enhances its molecular similarity to normal skin, potentially improving biointegration and clinical performance in burn care. These results contribute to understanding the positive effects of UGRSKIN applied to severely burnt patients.

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## *Optimizing clinical-grade MSC expansion: differential efficacy of platelet lysate and human plasma in adipose and bone marrow stem cells*

Pérez-Ortiz G1, Rejón-Camacho A1, Ávila-Fernández P1,2, Etayo-Escanilla M1,2, Aguilar-Pérez S1, González-Gallardo C1,2,3, Campos F1,2, Chato-Astrain J1,2, Alaminos M1,2, Martín-Piedra MA1,2, Garzón I 1,2

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### TEC-03 Ingeniería Tisular

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**Background:** Human mesenchymal stem cells (MSC) are increasingly used in tissue engineering protocols, due to their differentiation potential and proliferation capacity (1). For use in advanced therapies, bioartificial tissues must be fabricated using good manufacturing practices (GMP) guidelines, which strongly recommend the use of non-xenogeneic materials and reagents. However, current cell culture and tissue engineering protocols are largely dependent on the use of fetal bovine serum (FBS) supplementation. The use of this bovine product harbors several drawbacks, such as batch-to-batch variability, risk of disease transmission and potential immune reactions. One of the possible alternatives to the use of FBS is supplementation of the culture media with human-derived components, such as the human plasma (HP) and platelet lysates (PL). In this study, we compared the efficacy of HP and PL applied to the culture and expansion of two types of human MSC commonly used in tissue engineering for the generation of heterotypical models of the human oral mucosa, palate, cornea and skin by tissue engineering.

**Methods:** First, two types of human MSC were isolated from human tissue biopsies using enzymatic methods, as previously reported (2). Then, cells were cultured for several cell passages using DMEM medium supplemented with different concentrations of HP or PL, using the same medium with FBS as a control gold-standard group. Results were analyzed to determine the effects of each culture condition on cell survival, as determined by LIVE/DEAD analysis and cell proliferation.

**Results:** In general, all culture conditions allowed the efficient growth and proliferation of the MSC cultures, with more than 90% cell viability in all cases, suggesting that the use of HP and PL might support cell viability in a comparable manner to FBS. Cell proliferation varied among groups, with PL showing the highest rates of cell proliferation, supporting cell passaging without any detectable alterations.

**Conclusions:** The use of LP and PH is a safe promising alternative to FBS for the clinical-grade expansion of human MSC, fulfilling the requirements for tissue engineering translational products. Future studies should determine the real potential of these xenogeneic-free methods to generate bioartificial substitutes of the human oral mucosa, palate, cornea and skin generated with alternative MSC cell sources.

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## *Development of bioartificial tissues functionalized with fresh human bone particles for oral mucosa and palate tissue engineering applications*

Martín-Piedra MA1,2, España-López A3, Etayo-Escanilla M1,2, Pérez-Ortiz G1, Ávila-Fernández P1,2, Sánchez-Porras D1,2, Ortiz-Arrabal O1,2, García-García OD1,2, Fernández-Valadés R1,2,4, Campos F1,2, Garzón I1,2

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**TEC-03 Ingeniería Tisular**

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**Introduction:** Tissue engineering is focused on the design and development of biomimetic substitutes capable of promoting tissue regeneration in complex environments such as the oral cavity and, specifically, in those situations where a bone defect is present (1), such as cleft palate (2). This research aimed to develop and characterize an artificial oral mucosa substitute based on fibrin-agarose hydrogels functionalized with living human bone particles able to partially reproduce the structure of the oral mucosa in contact with palatal bone, such as the human hard palate, and to evaluate these functionalized oral mucosa substitutes at different levels.

**Methods:** A substitute of the human oral mucosa lamina propria was generated using fibrin-agarose hydrogels with oral fibroblasts immersed within. These substitutes were functionalized with small fragments of the human maxillary bone obtained from patients subjected to dental implants. These artificial tissues were kept in culture under controlled conditions for three weeks and evaluated at different times. Histological analyses were performed to assess morphological structure using hematoxylin-eosin and the presence of key extracellular matrix (ECM) molecules was assessed by histochemistry.

**Results and conclusions:** Functionalized human oral mucosa substitutes showed a progressive colonization of cells that migrated from the bone particles to the biomaterial after two weeks of culture, suggesting that the cells in bone particles remains viable and were able to proliferate and migrate ex vivo. These artificial tissues also showed some non-fibrillar components of the ECM at the extracellular compartment, suggesting that the cells in the artificial tissue were able to synthesize and release key ECM components, although the artificial lamina propria was still immature and not fully biomimetic to the native tissue. These findings support the potential of fibrin-agarose oral mucosa substitutes functionalized with bone particles as a promising strategy for short-term development of constructs with improved potential to integrate at bone surfaces, such as the hard palate, although extended maturation times may be required to achieve full matrix organization suitable for clinical application in oral regenerative therapies.

**Acknowledgements:** Supported by Instituto de Salud Carlos III, Ministry of Science, Innovation and Universities, Grant FIS PI24/00006, and co-funded by the European Union.

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## *Development of cryopreservation protocols for long-term storage of human artificial tissues generated by tissue engineering*

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### TEC-03 Ingeniería Tisular

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**Introduction:** Severe conditions affecting human tissues with limited regeneration potential are very difficult to manage, and the use of tissue grafts is often necessary. Tissue engineering offers an alternative strategy based on the generation of tissues consisting of an artificial stroma and an overlying epithelium. However, the half-life of these artificial tissues is very short. In this scenario, cryopreservation becomes an appealing option (1), as it may enable large-scale production, long-term storage and rapid clinical deployment of engineered constructs in patients with severe tissue defects. The present study analyses the efficiency of different cryopreservation protocols and how these protocols may influence the metabolic activity of artificial tissues, emphasising on their proliferative potential and their ability of matrix remodelling.

**Methods:** Bilayered artificial tissues containing stroma and epithelial layers were engineered using fibrin-agarose scaffolds. These artificial tissues were then cryopreserved in different cryoprotective agents containing DMSO, glycerol, trehalose or other cryopreservation compounds, and stored at three temperature conditions (4°C; -20°C; -80°C) for increasing periods of time. Two control groups were included: non-cryopreserved fresh artificial tissues (CTR+) and artificial tissues cryopreserved in PBS (CTR-). After thawing, immunohistochemical assays were performed to quantify total cell content and the percentage of cells showing positive expression for proteins related to cell proliferation and tissue remodelling.

**Results and conclusions:** In general, all cryopreservation agents were partially successful in terms of tissue preservation and cell proliferation, although none of them were fully functional. We observed trehalose showed appropriate results at 4° C. Nevertheless, at lower temperatures (-20 and -80°C), other reagents were more efficient and kept the proliferative status of the cells. Regarding matrix remodelling, we found a decrease at the longest periods of time. These findings suggest that cryopreservation could be a valuable option, and optimization of the protocols showing the most favorable preliminary results could contribute to develop more efficient cryopreservation methods for long-term storage of human bioartificial tissues.

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## *Characterization of novel natural biomaterials for corneal tissue engineering*

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### TEC-03 Ingeniería Tisular

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**Background:** Different types of biomaterials have been used to reproduce the structure of the human cornea by tissue engineering. Among others, natural biomaterials typically show adequate biocompatibility both ex vivo and in vivo (1) and are excellent candidates for use in corneal tissue engineering. In the present work, we have evaluated a transparent material obtained from marine invertebrates used as a scaffold biomaterial for cornea tissue engineering.

**Methods:** First, we obtained a natural biomaterial from the internal organs of marine invertebrates. This biomaterial was washed several times in PBS and evaluated histologically. Then, corneal epithelial cells were cultured on top of the biomaterial to determine the ex vivo biocompatibility of this biomaterial, and immunohistochemical analyses were carried out after 4 days of culture to determine cell phenotype.

**Results and conclusions:** We first found that the natural biomaterials were very transparent, and let most of the incoming visible light go through the biomaterial without alterations. Then, histological analyses found that the biomaterial was very homogeneous, and consisted of multiple parallel layers of material containing limited amounts of collagen. When cells were cultured on these biomaterials, we found a good integration, with cells forming an epithelial cell layer on top with positive expression of corneal crystallins, with cells showing high viability. In general, these results suggest that these marine-derived natural biomaterials may have putative usefulness for use in corneal tissue engineering.

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