# 1st SCIENTIFIC MEETING 'PASSION FOR SCIENCE'

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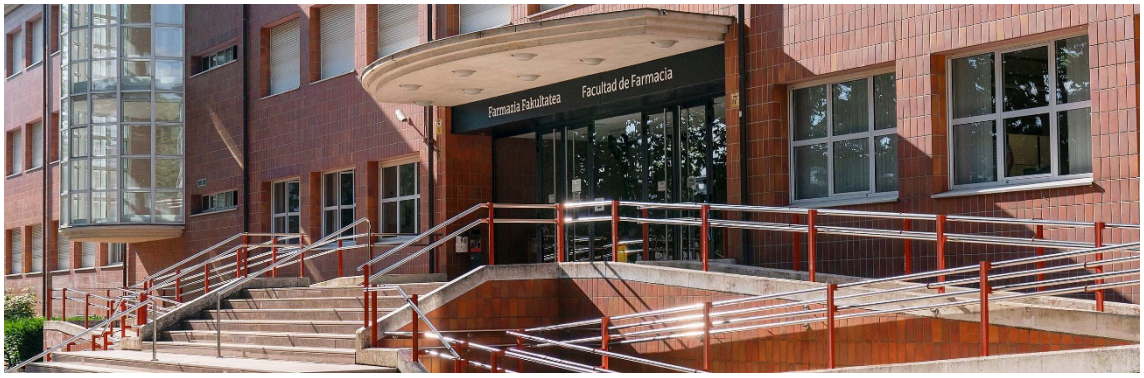


**19th November, 2024**

**FACULTY OF PHARMACY. UNIVERSITY OF THE BASQUE COUNTRY (UPV/EHU), Alava Campus, Vitoria-Gasteiz.**

**Organized by:**

"Aula de Medicina Personalizada UPV/EHU i+Med"

Faculty of Pharmacy (UPV/EHU)

**Sponsored by**:

Vice-Rectorate for Scientific-Social Development and Transfer and "Aula de Medicina Personalizada UPV/EHU i+Med"

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Jon Andrade (Cooperative member, i+Med)

## 1st SCIENTIFIC MEETING 'PASSION FOR SCIENCE'

**Location:** *Faculty of Pharmacy Auditorium (UPV/EHU). Vitoria-Gasteiz*

## PROGRAMME

### 19th November, 2024

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| 08:30 – 09:00 | **Registration & Presentations uploading/poster placement**<br>*Main hall of the Faculty of Pharmacy (UPV/EHU)* |
| 09:00 – 09:15 | **Welcome session**<br><br>**Dr. Manoli Igartua** (Vice-Rector of the Álava Campus, UPV/EHU)<br><br>**Dr. Mirari Ayerbe** (Dean of the Faculty of Pharmacy, UPV/EHU)<br><br>**Sandra Benito** (Laboratory Manager and Member of the Governing Council of the Cooperative of i+Med)<br><br>**Dr. Rosa Hernández** (Director of the Aula de Medicina Personalizada UPV/EHU i+Med) |
| 09:15 – 10:00 | **PLENARY SPEAKER I**<br><br>**Ana Beloqui.** Research Associate F.R.S.-FNRS & WELBIO Investigator at the Université Catholique de Louvain<br><br>*Exploiting the pathophysiology of the gut towards innovative oral peptide delivery strategies*<br><br>**Chairperson**: Edorta Santos-Vizcaino |
| 10:00 – 10:45 | **Oral presentations. Session I**<br><br>**Selected talks**: 7-minute presentation, 3 minutes for Q&A<br><br>**Chairperson**: Edorta Santos-Vizcaino<br><br>O1: Establishing a Scalable and Reproducible Framework for Using Extracellular Vesicles as Biologic Drug Delivery Vehicles. Zuriñe Eraña-Perez.<br><br>O2: Nanoparticle functionalization for targeted drug delivery. Ainhoa Goenaga. |

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| | O3: Combined Gene Therapy for Fabry Disease: Golden Lipid Nanoparticles as nanomedicines for siRNA and pDNA co-delivery. Marina Beraza Millor.<br><br>O4: Generation and characterization of patient-specific neurogenic 3D spheroids from human Dental Pulp Stem Cells for disease modeling and therapies. Jon Luzuriaga. |
| 10:45 – 11:30 | **Coffee break and Poster viewing** |
| 11:30 – 12:15 | **PLENARY SPEAKER II**<br><br>**José Luis García Cordero**. Head of Microtechnologies Group. Roche Institute of Human Biology. Basel.<br><br>*Organoid-on-a-chip technologies at Roche's Institute of Human Biology.*<br>**Chairperson**: Lourdes Basabe-Desmonts |
| 12:15 – 13:00 | **Oral presentations. Session II**<br><br>**Selected talks**: 7-minute presentation, 3 minutes for Q&A<br><br>**Chairperson**: Lourdes Basabe-Desmonts<br><br>O5: Microfluidic passive device for plasma enriched in platelets separation from whole blood made of acrylic and tape. Pablo E. Guevara-Pantoja.<br><br>O6: Paper-based microfluidic detection and quantification of a stress response indicator in crops: the microrna408. F. Nicolas Nazar.<br><br>O7: Autonomous microfluidic device for the naked-eye detection of benzodiazepines in adulterated beverages. Isabel Poves Ruiz.<br><br>O8: Study of the effect of the fungus Candida albicans in B16-F10 melanoma cells. Leire Aparicio Fernandez. |
| 13:00 – 14:30 | **Lunch Break and Poster Viewing** |
| 14:30 – 15:15 | **PLENARY SPEAKER III**<br><br>**Zaira García Carvajal.** Senior Researcher, i+Med<br><br>*Development of polymeric hydrogels and the use of nanotechnology for the encapsulation of molecules in health applications* |

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| | **Chairperson**: Jon Andrade |
| 15:15 – 16:00 | **Oral presentations. Session III**<br><br>**Selected talks**: 7-minute presentation, 3 minutes for Q&A<br><br>**Chairperson**: Gontzal García del Caño<br><br>O9: Development of hyaluronic acid injectable hydrogels based on thiol chemistry. Luis Pérez Pérez<br><br>O10: Development of a broad-spectrum SARS-CoV-2 nanovaccine focused on highly conserved S2 regions. Madalen Arribas Galarreta.<br><br>O11: First advances in the characterization of the biofilm composition of Arcobacter butzleri. Adrián Salazar-Sánchez.<br><br>O12: Beneficial effect of Opuntia extracts on oxidative stress and inflammation in a model of murine diet-induced liver steatosis. Irene Besné-Eseverri. |
| 16:00 – 16:45 | **Research career perspective and how to access an ERC grant**<br><br>**Ana Beloqui.** Research Associate F.R.S.-FNRS & WELBIO Investigator at the Université Catholique de Louvain<br>**Aitor Maestro** Posdoctoral Researcher at Organic Chemistry II Department (UPV/EHU) |
| 16:45 – 17:30 | **Oral presentations. Session IV**<br><br>**Selected talks**: 7-minute presentation, 3 minutes for Q&A<br><br>**Chairperson**: Javier Vicario<br><br>O13: The azo-Povarov cycloaddition, a potential bioorthogonal reaction. Xabier Jiménez-Aberásturi.<br><br>O14: Phosphorated antiproliferative agents, a new alternative for cancer treatment. Alba Rodríguez.<br><br>O15: Enantioselective synthesis of dihydroquinoline-hydantoin hybrids and their applications as antiproliferative agents. Zuriñe Serna-Burgos<br><br>O16: Toward hybrid organic/inorganic interfaces: how carbon-based nanomaterials can promote reorganisation and boosted activity in neuronal networks. Denis Scaini. |

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| 17:30 – 18:15 | **Coffee-break and Poster Viewing** |
| 18:15 | **Closing Remarks and Award Ceremony** |

## POSTERS

1. Synthesis of aziridines via Joullié-Ugi reaction as potential therapeutic agents. J. Allende.
2. mTOR inhibition increases channel expression in a model of LQTS2 in vitro. Beatriz Sáenz-Díez Pérez.
3. Mechanical Stimulation of Hydrogel-based Scaffolds Using a Bioreactor: Development of a Support System for Tendon Tissue Engineering. Ruiz-Alonso Sandra.
4. Influence of physical characteristics of the design of orodispersible 3D printlets on their disintegration time. N. Paccione.
5. Development and evaluation of a bioink for the 3D bioprinting of in vitro chronic wound models. Mikel Salmerón.
6. Regulatory framework for 3D bioprinted corneal constructs: From design to market authorization. Hodei Gómez Fernández
7. Bioink Enrichment with Growth Factor-Loaded Nanoparticles: Enhancing Clinical Efficacy of Regenerative Scaffolds. Jorge Ordoyo-Pascual.
8. Addition of Gamma-tocopherol rich Extract: impact on sunflower oil degradation process at frying temperatures. Susana Abrante Pascual.
9. Development of hydrogen suflide-activated theranostic prodrugs for selective cancer treatment through tetrazine dynamic chemistry. Oier Encinas.
10. Simple synthesis of 1,2-dihydroquinoline/hydantoin hybrids and the evaluation of their anticancer properties. Ainara Pellicer.

11. Determination of the MBIC and MBEC of amikacin, aztreonam and meropenem in Pseudomonas aeruginosa biofilms. Ohiana Rodríguez Medina.
12. Gene therapy for dry age-related macular degeneration with ionizable nanovectors: mRNA vs Pdna. P. Fernández-Muro.
13. Synthesis of nitrogen heterocyles as human topoisomerase i inhibitors. Leyre López de Aguileta.
14. Towards alternative diagnostics tools for fungal infections in patients with Cystic Fibrosis. L. Martin-Souto.
15. The importance of health care in the preferred-language chosen by the patient from a quality perspective. Jon Zarate.
16. Potential anti-obesity effects of three different microalgae extracts. Helen Carr-Ugarte.
17. Antimicrobial and biosurfactant production by halophilic bacteria. Elena Valgañón-Pérez.
18. 3D bioprinting of a novel graphene-based (bio)ink for tissue engineering. Irene Díez Aldama.
19. Investigating T cell activation through cytokine secretion using a novel cell monitoring platform. Sara Lpz. de Lacalle-Arregui.
20. Real-time visualisation of biochemical streptavidin-biotin interaction using gold nanoparticles and fiber optic. Sukayna Ezquerro Berdouzi.
21. Alginate/TiO2 Bead Biosystem for Cholesterol Determination in a Microfluidic device. Juncal A. Alonso-Cabrera.
22. Synthesis of functionalized chitosan polymeric nanoparticles and their properties for controlled drug release. Nekane Martin Mendia.
23. Showcasing the potential of different pharmacokinetic modeling approaches for the optimization of antimicrobial therapy. Ana Alarcia-Lacalle.
24. DUBs substrates: deciphering the UCH family. Erik P. Barrio.
25. Advances in Cell Affinity Assays with SCADA: Simple and Rapid Analysis of Cell-Molecule Interactions. Asier Inchaurraga Llamas.

26. Development of an Alginate Bead Biosystem for the Visual Detection of Cortisol. Sepideh Izaddoust.
27. Assessing Breast Cancer in vitro through Smart Bioelectronics. Gema del Rocío López-Buenafé.
28. New frontiers in antimycolata agent discovery: modification of known antibiotics. Angela Trejo Nogales.
29. Effects of Opuntia extracts. A comparison between epididymal and subcutaneous adipose tissue in a murine model of obesity. Iker Gómez García.
30. Viable Lactobacillus rhamnosus GG shows superior prevention over its inactivated variant on high-fat high-fructose diet-induced MAFLD prevention in rats. Laura Arellano García.
31. Food insecurity and health outcomes among the Spanish sub-cohort of the FINESCOP study. L. Garcia-Iruretagoyena.
32. A Novel Method for Advancing Bispecific Antibodies. Arkaitz Cano.
33. Functional stability of tetracycline: practical information for its use in bacterial selection. Adrián Salazar-Sánchez.
34. Construction and characterization of pIMM24, the first Escherichia coli – Arcobacter butzleri shuttle vector. Adrián Salazar-Sánchez.

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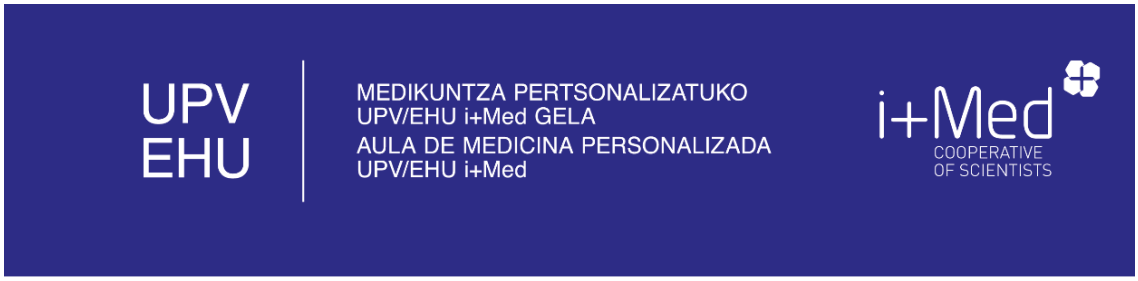


## PLENARY SPEAKERS

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## PLENARY SPEAKER I

Dr. Ana Beloqui is an Associate Professor and Research Associate from the Belgian Fund for Scientific Research (F.R.S.-FNRS) at the Université catholique de Louvain (UCLouvain, Brussels, Belgium), and group leader in the Advanced Drug Delivery and Biomaterials lab at the Louvain Drug Research Institute. Dr Beloqui's main research interests are the investigation of the oral drug delivery route (especially focused on the oral delivery of biologics) and the interaction of the drug delivery system with the gastrointestinal epithelium. She has received multiple scholarships and prizes, including the Galien Prize 2021 in Belgium, the Endocrinology, Diabetes and Metabolism Prize 2023 from the Belgian National Funds of Research, the Rising Woman in Science award from the Controlled Release Society in 2024, and the prestigious ERC starting and proof of concept grants from the European Research Council. She also serves as Associate Editor for the Journal of Controlled Release.

# Exploiting the pathophysiology of the gut towards innovative oral peptide delivery strategies

A. Beloqui[1,2]*
[1]*Advanced Drug Delivery and Biomaterials group, Louvain Drug Research Institute, Université catholique de Louvain, 1200, Belgium*
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## Abstract

Enteroendocrine cells (EECs), scattered along the gastrointestinal tract, respond to an ingested meal secreting a wide variety of gut hormones. Nutrients, such as lipids, can interact with various G-protein coupled receptors (GPCRs) expressed on EECs and induce the release of gut hormones [1]. Lipids are widely present in a wide range of excipients used in nanocarriers. My group has been working on the stimulation of gut hormones secretion via lipid nanocarriers orally administered [3-4]. The goal of this strategy is to stimulate the secretion of gut hormones while simultaneously providing with plasmatic levels of the encapsulating peptide towards an oral delivery alternative to the subcutaneous peptide injection. This represents a novel promising strategy for the treatment of gastrointestinal disorders.

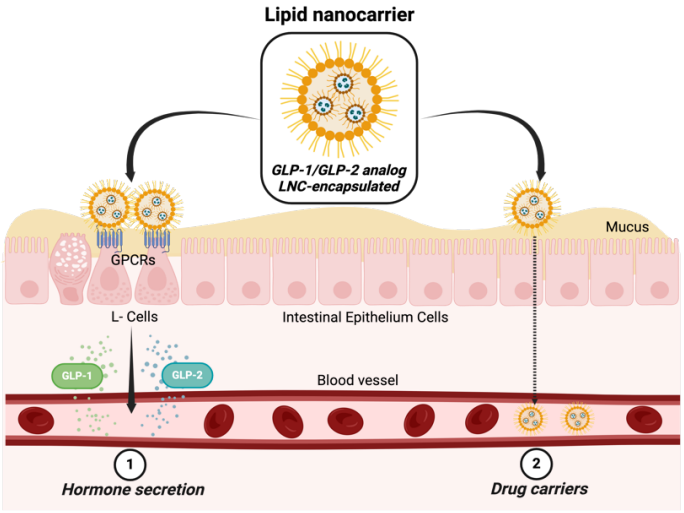


*Fig 1*. Schematic representation of the dual-action strategy via lipid nanocarriers for oral GLP-1/GLP-2 delivery [5].

## Acknowledgments

This work was supported by the FRS-FNRS (conventions J.0220.16, T.0013.19 and J.0009.22), FRFS-WELBIO (with the support of the Wallon region) (under grant agreement WELBIO-CR-2022 S–01) and the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No.850997-NanoGut).

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## PLENARY SPEAKER II

Since 2021, Jose L Garcia-Cordero has been leading the Microtechnologies group at Roche's Institute of Human Biology in Basel, Switzerland. At Roche, Jose's group is spearheading the development of microfluidic platforms to automate the culture of organoids and new microphysiological systems. From 2013 to 2021 he was a principal investigator at Cinvestav-Monterrey where his group was developing point-of-care diagnostics and microfluidic devices for single cell analysis. He was also an Adjunct Assistant Professor at the Dept. of Biomedical Engineering of the Mayo Clinic from 2018 to 2022 and a Visiting Professor at ETH Zurich from 2019 to 2020. He obtained a PhD under the guidance of Prof. Tony Ricco at DCU (Ireland) and was a postdoc with Prof. Sebastian Maerkl at EPFL in Lausanne Switzerland.

# Organoid-on-a-chip technologies at Roche's Institute of Human Biology

Jose L Garcia-Cordero

[1]*Institute of Human Biology, Pharma Research and Early Development (pRED), Roche Innovation Center Basel, 4070, Basel Switzerland*

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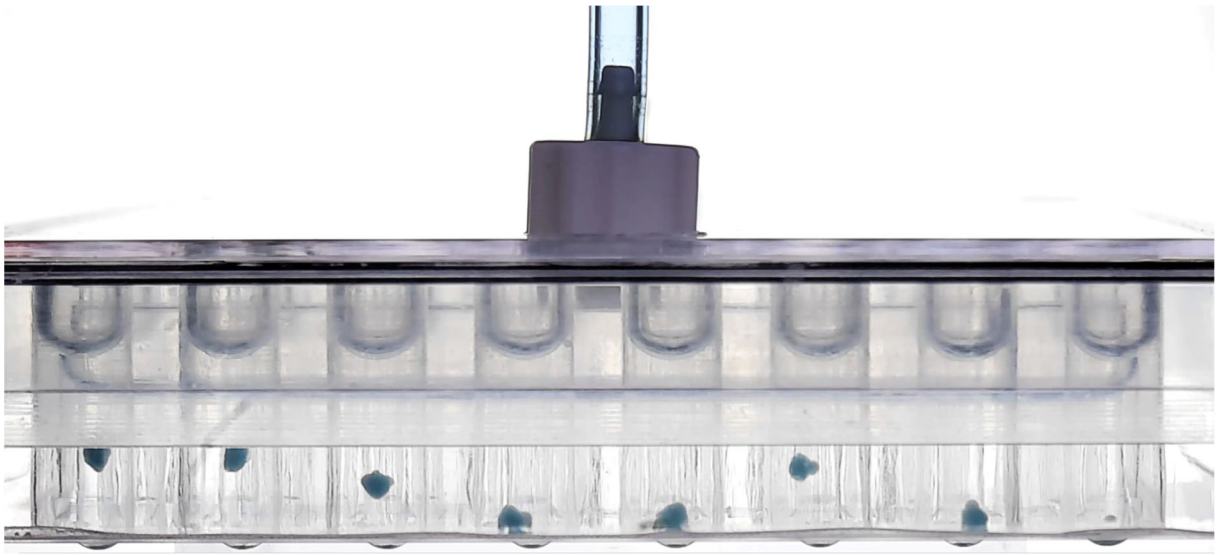
**Abstract**

In this talk, I will present the Institute's efforts to develop in vitro human models based on organoids and organ-on-a-chip technologies. I will discuss the current challenges in the pharmaceutical industry regarding the development of new drugs and how these new microfluidic-based technologies can help decrease costs and accelerate drug discovery. I will highlight two new technologies developed at Roche's Institute of Human Biology: mini-colons produced in a microfluidic device using tissue engineering, and the PulsePlate, a platform the size of a well plate designed to culture different types of organoids in suspension. For both technologies, I will describe the fabrication methods, characterization, and biological results. I will conclude with a perspective on the organ-on-a-chip field



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## PLENARY SPEAKER III

Zaira García Carvajal was born Guadalajara, Jalisco, Mexico. She is a staff member as a Research Professor at Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, AC (CIATEJ). Zaira is a pharmacist with a master s degree in industrial and Galenic Pharmacy and a PhD in Pharmaceutical Technology (Complutense University of Madrid/Madrid Institute of Materials Science of the Higher Council for Scientific Research of Spain). She worked in the pharmaceutical manufacturing industry in Mexico and Spain. Her scientific research is focused on designing and developing polymeric hydrogels for health applications (tissue engineering and sustained release) using nanotechnology and 3D printing. She belongs to the National System for Evaluating Toxicity of Nanomaterials of Mexico (SINANOTOX). She has co-supervised several bachelor's, master's and doctoral theses. She is the author and co-author of over 40 original review articles and chapters in internationally peer-reviewed journals and books. She is the inventor and co-inventor of 10 patents (applied and granted) in health materials. It has technological transfer to the veterinary pharmaceutical sector. She is involved in open and inclusive science projects for science divulgation.

*I SCIENTIFIC MEETING "PASSION FOR SCIENCE", 2024*

**Development of polymeric hydrogels and the use of nanotechnology for the encapsulation of molecules in health applications**

Zaira Y. García Carvajal*[1,2], Moisés Martínez [2], BioMaterials Lab members[2]

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2. *Unidad de Biotecnología Médica y Farmacéutica, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C. (CIATEJ), Guadalajara 44270, México*

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**Abstract**

The pharmaceutical, medical and food industries are paradigmatic examples of the exciting transformation of the world economy, which has grown considerably in recent years, generating innovative products based on nanotechnology [1]. Moreover, hydrogels are one of the most widely investigated and versatile technologies for drug delivery and tissue engineering applications [2], [3] [4]. Basic science research carried out at CIATEJ focused on the use of hydrogels for biomedical applications will be shown: 1) cartilage and bone tissue engineering, 2) cell spheroids formation, 3) oral and vaginal delivery systems and 4) polymer-biogenic nanoparticle composites.

**Keywords:** hydrogels, scaffolds, nanomaterials, biopolymer

**Graphical abstract:**

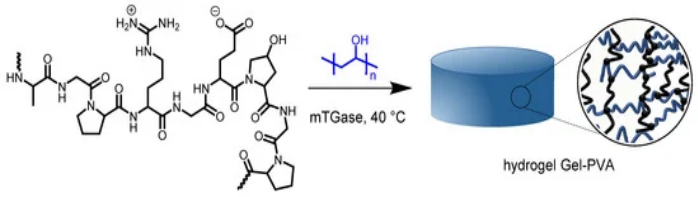


*Fig 1. Pathway to prepare the Gel–PVA hydrogel. Microbial transglutaminase acts as a crosslinking agent.*

**Acknowledgements**

The research projects partially supported by FORDECYT-PRONACES/490754/2020 and SSA/IMSS/ISSSTE-CONACYT" under grant 234073

**References**

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# ORAL COMMUNICATIONS

# Establishing a Scalable and Reproducible Framework for Using Extracellular Vesicles as Biologic Drug Delivery Vehicles

Z. Erana-Perez[1,3]*, M. Igartua[1,2,3], E. Santos-Vizcaino[1,2,3] and R.M. Hernandez[1,2,3]

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[2]*Biomedical Research Networking Centre in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN).*

[3]*Bioaraba, NanoBioCel Research Group, Vitoria-Gasteiz, Spain.*

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**Abstract**

Extracellular vesicles (EV) represent a cutting-edge platform for delivering biologic drugs, including proteins and genetic material, due to their natural ability to mediate intercellular communication [1]. EV can be loaded by endogenous methods, which streamline drug manufacturing and loading processes by genetically engineering EV-producer cells to naturally produce and package therapeutic biomolecules directly into EV. However, challenges such as homogeneity and the lack of standardized protocols limit the translation of endogenously-loaded EV into clinically viable pharmaceuticals [2]. To overcome these limitations, we have developed a comprehensive workflow for the clinical application of EV-based drug delivery systems, focusing on reproducibility, process control, and industrial scalability, all while aligning with MISEV 2023 guidelines [3] to enhance comparability across studies. First, we have established a stably engineered cell line that overexpress erythropoietin (EPO). We then isolated EPO-loaded EV via differential centrifugation, which enables large-volume processing while maintaining purity. We conducted a rigorous characterization process to assess membrane integrity, size distribution, and surface marker expression, ensuring batch-to-batch consistency. Additionally, a key component of our framework is that we have designed of a robust in-house quality control assay to ensure purity and elimination of contaminant soluble proteins in EV formulations. Then, we have systematically evaluated both EPO protein and EPO mRNA content within EV to ensure reproducible loading outcomes. Finally, we have assessed the biological activity of EPO-loaded EV through *in vitro* and *in vivo* assays. Overall, our framework established a scalable and standardized approach for EV-based drug delivery, addressing key barriers in the clinical translation of biologic therapies.

**Keywords:** extracellular vesicles, biologic drug delivery, quality control framework, clinical translation.

**Graphical abstract:**

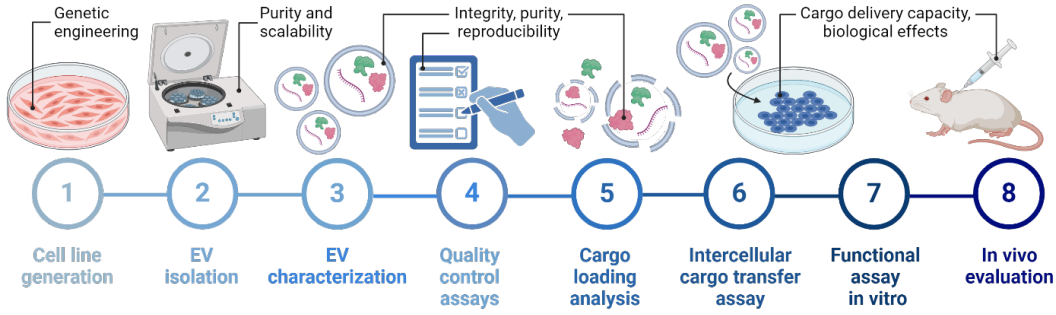


*Fig 1*. Schematic representation of the established framework

**Acknowledgments**

This project was partially supported by the Basque Government (Consolidated Groups, IT1448-22). Z. Erana-Perez thanks the Basque Government for the PhD grant (PRE_2023_2_0253).

**References**

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# Nanoparticle functionalization for targeted drug delivery

Ainhoa Goenaga[1], Arkaitz Cano[2], Ana del Pozo[1,3], Marian Solinis[1,3], Alicia Rodriguez[1,3], Edurne Rujas[1,3,4,5], José L. Nieva[4,6], Beatriz Apellaniz[2,3]

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**Abstract**

Currently, monoclonal antibodies are key in cancer treatment: their recognition of tumor-associated antigens stimulates a specific immune response that is able to eliminate cancer cells or interferes with signaling pathways crucial for their survival and proliferation. Within the last decades, these antibodies have been engineered to carry cytotoxic drugs to achieve selective delivery, minimizing off-target toxicity and broadening the therapeutic window of chemotherapeutic agents(1). The antitumor efficacy is often correlated to the quantity of drugs delivered by the antibody. Hence, to enhance the amount of drug delivered to the site of action, in this study we employed a maleimide chemical reaction to conjugate a model antibody, commonly employed in cancer treatment, with a nanostructured lipid carrier (NLC). These nanoparticles are comprised of an unstructured solid lipid matrix made of a mixture of blended solid and liquid lipids and an aqueous phase containing a surfactant or a mixture of surfactants able to transport poorly soluble compounds(2). Specifically, we demonstrate that the resulting particles are monodisperse (with a PDI of 0.27), have a hydrodynamic radius of 140 nm, and exhibit high specificity for the target epitope. Additionally, these particles exhibited high stability over time. Our current objective is to load these nanoparticles with the antitumoral drug topoisomerase I inhibitor SN-38, and demonstrate the capacity of these particles to efficiently target tumor cells.

**Keywords:** antibody, NLC, drug delivery.

**Graphical abstract:**

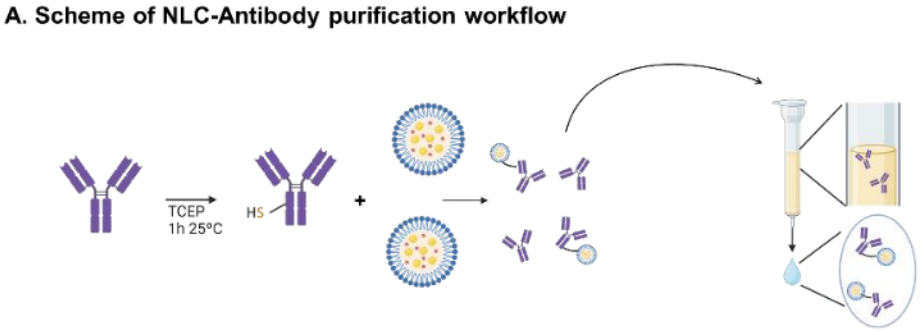


*Fig 1*. NLC-Ab conjugation is accomplished by reduction of the antibody by TCEP for 1 h, following the addition of NLC. After incubating the sample, free Ab is separated from the NLC-Ab sample by a CaptoCore400 resin via gravity flow separation.

# Combined Gene Therapy for Fabry Disease: Golden Lipid Nanoparticles as nanomedicines for siRNA and pDNA co-delivery

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**Abstract**

Fabry disease (FD) is a multisystemic rare disorder caused by mutations in the *GLA* gene encoding α-Galactosidase A (α-Gal A) enzyme. Consequently, there is a progressive lysosomal accumulation of glycosphingolipids, especially globostriaosylceramide (Gb3) and its derivate lyso-Gb3 [1]. Current therapies, based on enzyme replacement therapy and oral chaperones, show limitations in terms of costs and efficacy. Gene therapy offers different strategies with revolutionary potential for the treatment of pathological conditions that lack effective treatments. Gene supplementation therapy of a healthy copy of the *GLA* gene by plasmid DNA (pDNA) can promote the production of α-Gal A by natural processes of patients' native cells. In addition, genetic substrate reduction therapy (gSRT) by small interfering RNA (siRNA) to block the expression of Gb3 synthase enzyme (Gb3S) represents a new strategy for limiting Gb3 accumulation, especially relevant in mainly affected organs (kidneys, heart and nervous system). Nanomedicine allows the design of delivery nanosystems tailored to the type of nucleic acid, disease and target cell for effective and personalized treatments [2]. In this work, a novel nanomedicine based on lipid nanoparticles (LNPs) and gold nanoparticles (GNs) has been designed for the co-delivery of both pDNA and siRNA to address FD by combining gene supplementation therapy and gSRT. The composition of LNPs, the size of GNs, methods for the LNP preparation and the incorporation of all components and different ligands were deeply optimized. The nanomedicines based on golden LNPs prepared in this work, with particle size ranging from 200 to 300 nm and positive surface charge, efficiently bound, protected and released both nucleic acids. A high association of the golden LNPs to a cellular model of FD, IMFE-1 cells, was observed. The efficacy of the formulations depended on their final composition, however, all nanomedicines provided an increase in α-Gal A activity, a significant reduction of Gb3S mRNA expression (detected by RT-qPCR and immunocytochemistry) and most of them induced the reduction of Gb3 for at least 15 days after transfection. In conclusion, nanomedicine based on golden LNPs designed with the ability to combine and appropriately release different types of nucleic acids enables the application of different gene therapy strategies simultaneously in the same cell, broadening the current therapeutic perspectives for addressing FD by gene therapy.

**Keywords:** Fabry disease, Gene therapy, Genetic substrate reduction therapy, Lipid nanoparticles.

**Graphical abstract:**

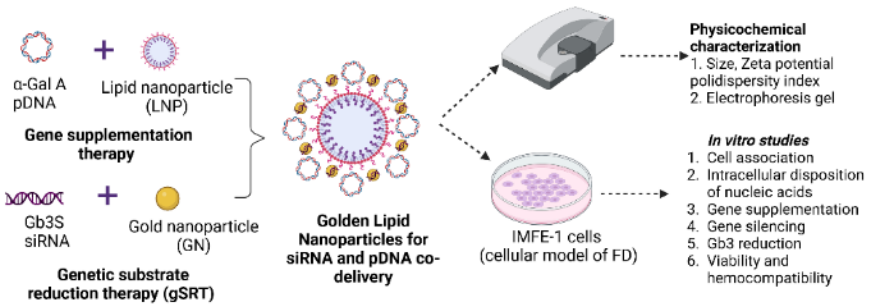


*Fig 1.* Combined nanomedicines containing pDNA and siRNA and their characterization.

**Acknowledgments**

This work was supported by MCIU/AEI/FEDER, UE (RTI2018-098672-B-I00) and the Basque Government (IT1587-22). M. Beraza-Millor and P. Fernández-Muro thank the UPV/EHU (PIF21/61 and PIFG23/45) and J. Rodriguez-Castejón thanks the Basque Government (PRE_2021_2_0221) for their research grants. The authors thank COST Action CA17103-Delivery of Antisense RNA Therapeutics (DARTER).

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# Generation and characterization of patient-specific neurogenic 3D spheroids from human Dental Pulp Stem Cells for disease modeling and therapies

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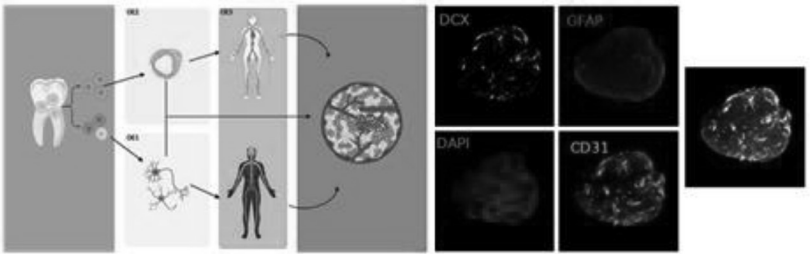
**Abstract**

The use of 3D brain organoids has become increasingly important as a more accurate in vitro model, offering enhanced structural and physiological fidelity compared to traditional 2D methods. Additionally, these organoids provide an alternative to animal models for studying neurological disorders and screening pharmacological therapies. Traditionally, brain organoids have been created from embryonic stem cells (ESCs) and/or induced pluripotent stem cells (iPSCs), using protocols to induce neural maturation. However, a major limitation of this model is the lack of vascularization [1], which restricts the size and overlooks vital cellular interactions necessary to study neurological pathophysiology and brain cell homeostasis, as well as the lack of specificity to the patient. In our innovative approach, we present a co-culture system designed to produce patient-specific neurogenic vascularized 3D spheroids. Taking advantage of the neurogenic and endotheliogenic capacity of human dental pulp stem cells (hDPSCs) [2, 3 ]. We demonstrate for the first time in this work that hDPSCs, a subset of human neural crest stem cells, can differentiate into neural precursors (GFAP- or DCX-positive cells) as well as endothelial cells (CD31 and vWF) within the generated 3D spheroids. We have established a correlation between the initial cell quantity and the resulting spheroid size, allowing for customization based on specific applications (e.g., brain organoid assembly, microfluidic devices).We document the first use of hDPSCs in 3D spheroids as a source of human vascular endothelial and neurogenic cells for creating 3D structures. Our model serves as proof of concept, paving the way for the generation of vascularized, mature 3D brain organoids from hDPSCs. Additionally, our vascularized spheroids could enable fully autologous generation of patient-derived brain microstructures, replicating vascular-neural interactions in 3D cultures and providing an alternative to in vivo animal testing.

**Keywords:** hDPSCs, Neurodifferentiation, Disease modeling. 3D spheroids.

**Graphical abstract:**




**Acknowledgments**

This research was supported by University of the Basque Country (UPV/EHU) grant COLAB22/07, the Basque Government (IT1751-22; to G.I.), the Health Department of the Basque Government (grants No. 2021333012 and 2023333035; to J.R.P.), grants PID2019-104766RB-C21 (J.R.P.), PID2023-152704OB-I00 (J.R.P. and G.I.) funded by MCIN/AEI/10.13039/501100011033 by the European Union (NextGenerationEU) "Plan de Recuperación Transformación y Resiliencia", and POLIMERBIO SL (UPV/EHU contract 2023.0012).

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# MICROFLUIDIC PASSIVE DEVICE FOR PLASMA ENRICHED IN PLATELETS SEPARATION FROM WHOLE BLOOD MADE OF ACRYLIC AND TAPE

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**Abstract.** We present a microfluidic passive device to separate plasma enriched in platelets from whole blood. The device uses the gravity sedimentation of blood cells and its capture in multiple trenches as a working principle [1]. The device is capable of processing 1 mL of whole blood in 45 min obtaining approximately 300 µL of plasma with at least 2-fold platelets concentration with 18.3% less platelet activation compared to conventional centrifugation. Our device can pave the way to obtain high-quality plasma enriched in non-activated platelets using a simple and low-cost method. The plasma separator is easy to operate, fabricate, assemble, and is adaptable to any application that needs plasma enriched in high-quality platelets.

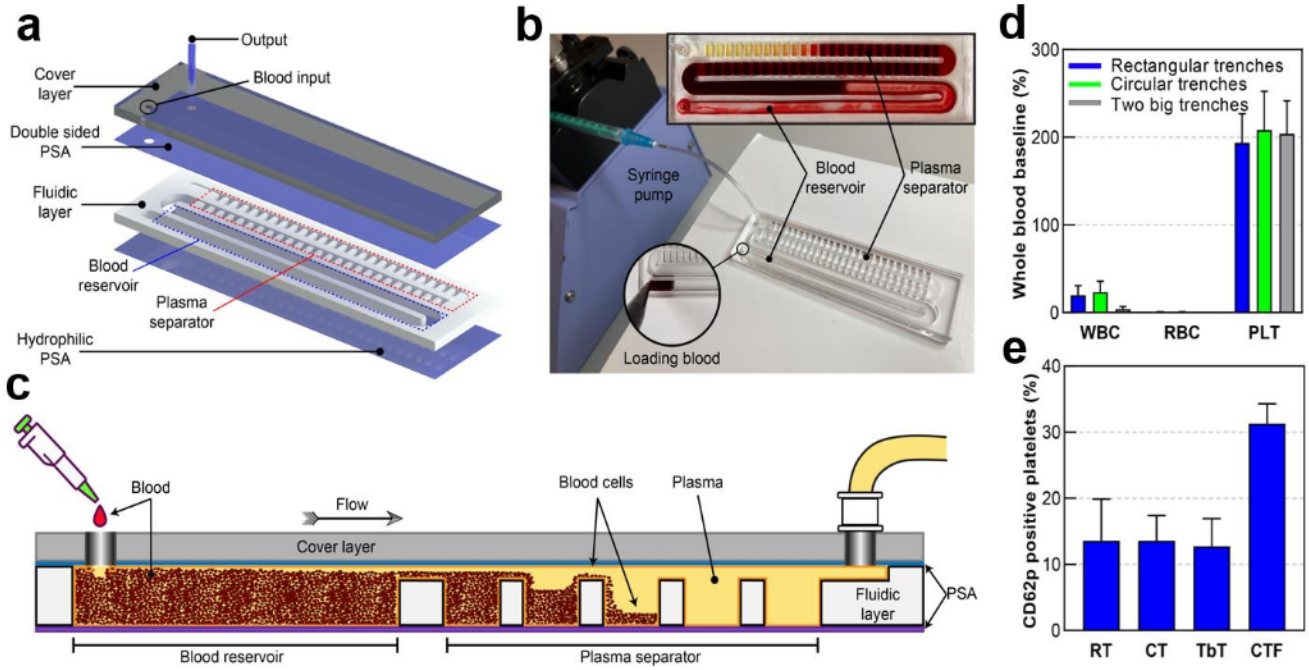


***Figure 1**: **a**) Exploded view of the device with rectangular trenches. **b**) Experimental setup. Inset: device separating plasma **c**) Schematic showing the sedimentation process along the microfluidic device. **d**) Blood analyzer results of plasma collected from the device for white blood cells (WBC), red blood cells (RBC) and platelets (PLT). N=3 Error: S.D. **e**) Summary of the platelets activation results for the rectangular (RT), circular (CT) and two big (TbT) trenches devices along with the centrifugation method (CTF). N=3 Error: S.D.*

**Acknowledgements**
We acknowledge funding support from Basque Government, under "Grupos Consolidados" with Grant No. IT1633-22 and the funding support from "Ministerio de Ciencia y Educación de España" under grant PID2020-120313GB-I00 / AIE / 10.13039/501100011033. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101034379

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# PAPER-BASED MICROFLUIDIC DETECTION AND QUANTIFICATION OF A STRESS RESPONSE INDICATOR IN CROPS: the microRNA408

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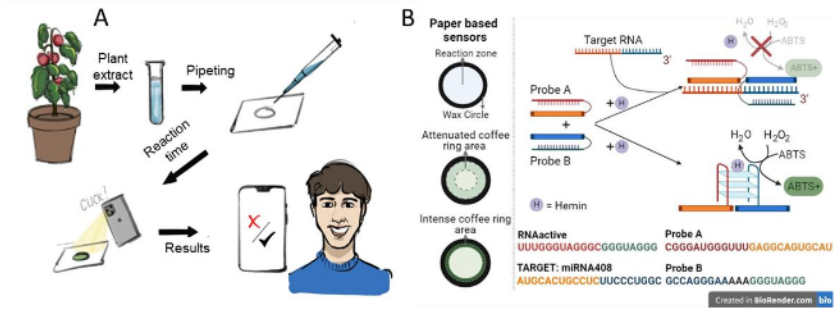
Early detection of stress mediators' in crops is crucial for optimizing agricultural production [1], aligned with long-term food security [2]. microRNAs (miRNAs) are key regulators of stress responses in crops which inform on physiological adaptive mechanisms [3]. RNAzymes, being RNA molecules with catalytic capability, can be designed to specifically bind to sequences of interest within a miRNAs pool. This binding generates a detectable colorimetric signal, allowing identification and quantification of target miRNAs [4]. Detection of miRNAs with RNAzymes offers a promising strategy to monitor crop' stress. We present the design, manufacturing and performance of a device for detecting stress response mediators in plants using RNAzymes based colorimetric sensors (graphical abstract, A). Specific RNA Sensing Probes (SP) were designed for miRNA408 (Target), a key stress marker in crops [3], (graphical abstract B). We designed paper-based sensors in the form of a circle area delimited by a wax ring where the reaction took place. Results indicate that the RNAactive (the active portion of the RNAzyme) functions properly, that the SPs have activity on their own and that the addition of the miRNA408 decreases the intensity of the colour signal [4]. Specifically, adding increasing amounts of target (0-640 ng), a greater inhibition of the system occurred, resulting in a larger attenuated coffee ring area (less colour) on our sensors (graphical abstract, B). Finally, this paper-based sensor has been integrated in a hybrid paper/plastic microfluidic device. As a proof-of-concept the device was tested with synthetic miRNA408 (640 ng) or vehicle (distilled water) for its colorimetric detection. Coherently, the presence of Target inhibits the reaction significantly, thus reducing the signal intensity in the detection area. These results constitute first steps in the design of point-of-need devices for early stress detection in crops, empowering farmers with strategies for early decision-making.

**Keywords:** precision agriculture, chemical sensors, µPAD, microfluidic device.

**Graphical abstract:**




***Microfluidic detection of a stress indicator in plants.*** *Panel A:* schematic representation of the detection process from tomato plant extract to results readout. *Panel B:* Schematic representation of RNAzymes system functioning. Paper based sensors are represented. RNAactive, Probes, and Target sequences are detailed.

**Acknowledgements**

These results are part of the PREDESTOM project (TED2021-129273B-C33), funded by the Ministry of Science and Innovation of Spain through the State Research Agency (MCIN/AEI/10.13039/501100011033); Recovery, Transformation, and Resilience Plan, and with NextGenerationEU. Also financed by the Department of Education of the Basque Government (Consolidated Research Group) under Grant IT1633-22.

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# Autonomous microfluidic device for the naked-eye detection of benzodiazepines in adulterated beverages

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**Abstract**

This study focuses on the development of a microfluidic paper-based analytical device (μPAD) for the on-site detection of benzodiazepines, frequently involved in drug-facilitated sexual assaults (DFSA). μPADs have recently emerged as a breakthrough technology for rapid, multiplexed analysis of a wide range of analytes at crime scenes [1, 2]. Scott's reaction, a complexation process using Cobalt (II) thiocyanate in an acidic medium, has been established as a universal colorimetric sensor for detecting benzodiazepines in solution but has yet to be adapted for use on a solid support or integrated into a microfluidic device [3]. Therefore, a multi-layered device, which incorporates polymeric materials and cellulose paper was developed and tested, demonstrating a limit of detection of 0.54 mg $mL^{-1}$ for midazolam.

**Keywords:** microfluidic device, benzodiazepines, colorimetric reaction, beverages.

**Graphical abstract:**

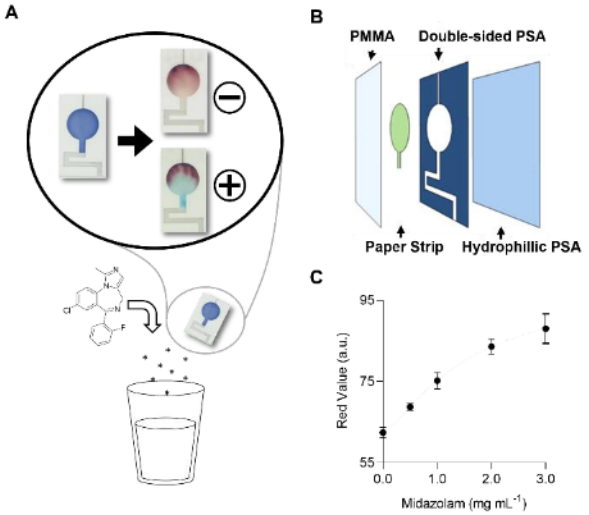


*Fig 1*. A) Scheme of the protocol followed for analysis. Pictures of the color changes in the presence and absence of midazolam. B) Scheme of the different layers forming the microfluidic device test. C) Calibration curve for midazolam using the microfluidic device.

**Acknowledgments**

Research and technical support from ALBA Biotech. We acknowledge funding support from Basque Government, under Grupos Consolidados with Grant No. IT1633-22 and Basque Government Predoctoral grant "PRE_2023_1_0061"; "Ministerio de Ciencia y Educación de España" under grant PID2020-120313GB-I00/AIE/10.13039/501100011033 and Spanish Government under the program "Margarita Salas" funded by "Unión Europea-Next Generation EU".

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# Study of the effect of the fungus *Candida albicans* in B16-F10 melanoma cells

L. Aparicio-Fernandez[1*], N. Cazalis-Bereicua[1], M. Areitio[1], O. Rodriguez-Ereñaga[1], L. Martin-Souto[1], I. Buldain[1], A. Benedicto[2], J. Márquez[2], B. Arteta[2], A. Pellon[3], D. L. Moyes[3], A. Aransay[4], N. Macias-Cámara[4], M. Gonzalez[4], J. Ezequiel Martin Rodriguez[4], J. Anguita[4, 5], A. Rementeria[1], A. Antoran[1] and A. Ramirez-Garcia[1]

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**Abstract**

Cancer is one of the leading causes of death worldwide. In recent years, interest in investigating the involvement of fungi in carcinogenesis has increased significantly, with *Candida albicans* standing out especially. However, to date, there are few studies on the effect that this fungus exerts on tumor cells and, specifically, none in the case of melanoma. Therefore, the objective of this work is to delve into the changes induced by *C. albicans* in melanoma cells, both *in vitro* and *in vivo*.

First, the effects of C. albicans on the pro-tumor phenotype of melanoma cells were evaluated through *in vitro* assays that included cell migration, adhesion to endothelial cells, metabolic reprogramming and cell proliferation, as well as its pro-metastatic effect through *in vivo* experiments in mice. The results indicated a significant increase in all processes after exposure to the fungus. To understand the underlying mechanisms, RNA-seq gene expression analyzes were carried out, which revealed the overexpression of genes linked to the MAPK and HIF-1 signaling pathways. To go even deeper, specific molecular inhibitors of different metabolic pathways were used, after which the secretion of the pro-angiogenic molecule VEGF was analyzed by ELISA, and the expression of several related proteins and genes was analyzed by Western blot and RT-qPCR. The results confirmed the importance of pro-inflammatory pathways mediated by the AP-1 transcription factor, mainly c-Fos, and of the metabolic reprogramming that *C. albicans* promotes in melanoma cells.

In conclusion, *C. albicans* induces changes that favor a more aggressive phenotype in melanoma cells, through the MAPK/AP-1 and HIF-1 pathways, important for the creation of a pro-tumor and metastatic environment. Therefore, the findings obtained in this work could allow the development of new therapies and treatments for cases of melanoma that could be caused or aggravated by *C. albicans*.

**Keywords:** *Candida albicans*, cancer, inflammation

**Acknowledgments**

This research was supported by Basque Government (IT1657-22). LAP, MA, LMS and ORE have received a predoctoral grant from Basque Government (MA, LMS and ORE) or UPV/EHU (LAP).

# Development of hyaluronic acid injectable hydrogels based on thiol chemistry

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**Abstract**

Hyaluronic acid (HA) is a non-sulphated glycosaminoglycan found naturally in the human body, such as connective tissue, skin, and synovial fluid. This polymer has been extensively studied to produce chemically cross-linked hydrogels, which allow the modulation of mechanical properties, the addition of new functionalities and the prolongation of residence time in the human body. They are promising candidates in various biomedical fields, for example as scaffolds for tissue engineering or carriers for drug or cell delivery [1]. Hyaluronic acid hydrogels have attracted attention in the las decades in the aesthetic field as volume fillers, where they stand out as the second non-surgical procedure in the world by plastic surgeons in 2023, with more than 5 million treatments [2][3]. Nowadays, there is a growing demand for injectable hydrogels as they can be administered through needles or small cylinder cannulae, thus avoiding invasive surgery. In this work, we optimized a methodology for the development of injectable granular hydrogels chemically crosslinked by thiol Chemistry [4]. To this end, hyaluronic acid was modified with thiol groups. Subsequently, macro-hydrogels were produced through disulfide bond formation and Michel addition type reactions using PEGs derivatives. Hydrogels were fully characterized as to their viscoelastic properties and hydrolytic stability. Finally, the material was mechanically fragmented and sterilized to produce injectable formulations. The resulting formulations exhibited characteristic properties such as shear thinning, recovery after deformation, microporosity and stability. This simple and inexpensive technique multiplies the applications of conventional hydrogels by allowing their injectability.

**Keywords:** Hyaluronic acid, Hydrogel, Injectability, Thiol Chemistry.

**Graphical abstract:**

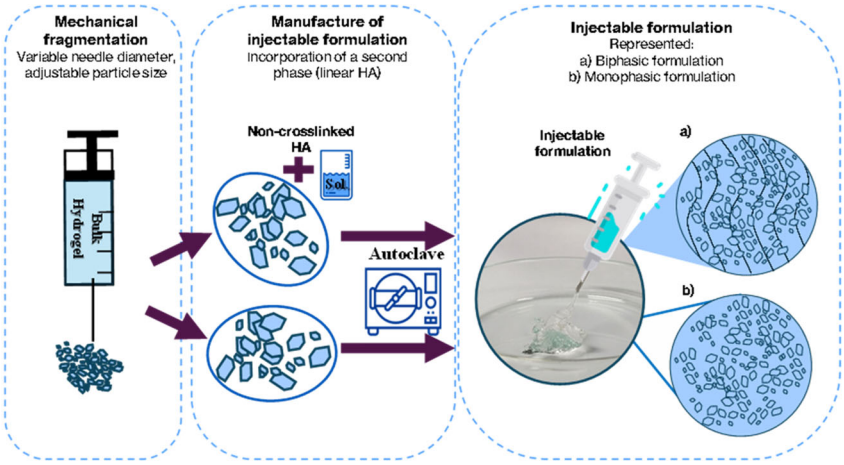


*Fig 1.* Manufacture of biphasic and monophasic hydrogels.

**Acknowledgments**

This work was supported by the Spanish AEI through the call 'Doctorados Industriales 2019' (DIN2019- 010868). We acknowledge financial support from the project Molecular Design of Polymers for Biomedical Applications (MEDIPOL. H2020-MSCA-RISE-2019. Grant agreement ID: 871650).

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# Development of a broad-spectrum SARS-CoV-2 nanovaccine focused on highly conserved S2 regions

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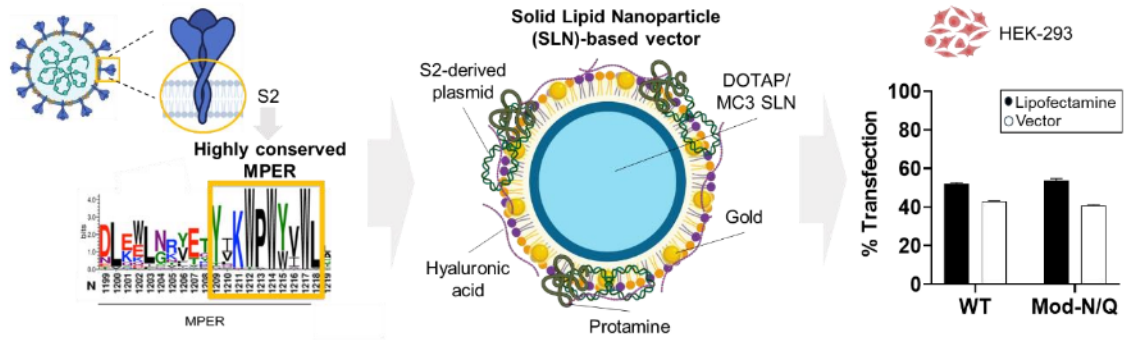
**Abstract**

4 years after the first cases of coronavirus disease, SARS-CoV-2 remains in circulation, exhibiting noteworthy genetic and antigenic evolution within its spike protein (S). Specifically, mutations are selected within the accessible S1 subunit[1]. Consequently, even if current Omicron XBB.1.5-based vaccines elicit cross-reactive responses against circulating variants, constant surveillance and updates of the antigens included in the vaccine platforms are needed. To circumvent this, we analyzed in a SARS-CoV-2 infected human cohort the immunogenicity of the membrane-proximal external region (MPER) of the S2 subunit, a highly conserved region across the Orthocoronavirinae subfamily. We report that, although weak, a portion of the patients did elicit antibodies against this region. Moreover, we characterized its structure in a low-polarity environment and in lipid membranes, and we showed its fusogenic potential. These results confirm its suitability as a vaccine target. Considering the impact of lipid membranes on the structure of the MPER, we assessed its expression in the membrane of eukaryotic cells. For that, we designed a wild type and a modified[2] S2-derived DNA sequences including the MPER, which were delivered through solid lipid nanoparticles. These results support the feasibility of designing a nucleic-acid vaccine targeting this region. Currently, we are assaying the designed and optimized vaccine platform *in vivo* in mice.

**Keywords:** SARS-CoV-2, nanovaccines, antiviral immunity, MPER.

**Graphical abstract:**




**Acknowledgments**

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# First advances in the characterization of the biofilm composition of *Arcobacter butzleri*

Adrián Salazar-Sánchez[1,2,*], Leire Abad-Zubia[1], Lorena Laorden[1,2], Rodrigo Alonso[1,2], Ilargi Martínez-Ballesteros[1,2], Irati Martinez-Malaxetxebarria[1,2]

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## Abstract

*Arcobacter butzleri* is a foodborne human pathogen causing diarrhoea and enteritis. Its ability to form biofilms facilitates its spread along the food chain [1]. However, the composition of its biofilm is currently unknown. This work aims to discover it, contributing to the knowledge of this species and facilitating its control in the future. To obtain the biofilm, the extracellular polymeric substances of the strains RM4018$^T$ and P8 were extracted, and their carbohydrates, proteins, nucleic acids, and lipids were quantified using 2-phenoxyethanol assay, BCA and Bradford methods, Nanodrop, and sulfo-phospho-vanillin assay, respectively. Additionally, FTIR analyses were perform on the extract. Currently, each part is being extracted and characterized by GC-MS and/or HPLC. The results indicated that RM4018$^T$ and P8 produce biofilm biomass of approximately 1.79 ± 0.28 and 1.81 ± 0.48 mg/100 mL broth, respectively. The carbohydrate and lipid amount was found to be <0.02% for both strains, while the protein content was 5.25 ± 3.44% and 4.79 ± 1.99%, respectively. The nucleic acid content was 2.11 ± 0.60% and 2.53 ± 0.98%, respectively. The FTIR analysis revealed the presence of groups that could correspond to glucosamine-like substances, which may explain the low quantification of carbohydrates by the employed method. Unfortunately, GC-MS and HPLC results are not yet available. Apparently, the biofilm of *A. butzleri* could be mostly composed of polysaccharides, with a limited presence of proteins and nucleic acids and virtually no lipids.

**Keywords:** *Arcobacter butzleri*; Biofilm matrix; Biofilm composition.

## Graphical abstract:

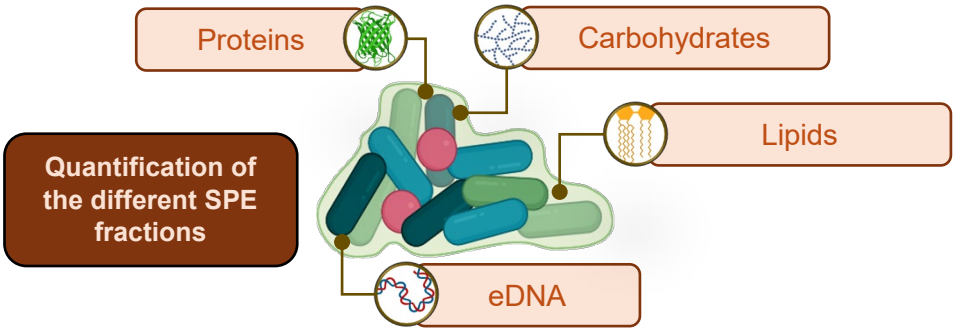


*Fig 1*. Methodology employed for the biofilm characterization.

## Acknowledgments

This work was supported by the project GIU21/021 (UPV/EHU). A.S.-S. has a PhD grant from the UPV/EHU.

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# Beneficial effect of *Opuntia* extracts on oxidative stress and inflammation in a model of murine diet-induced liver steatosis

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**Abstract**

Oxidative stress and inflammation are involved in the development of metabolic dysfunction-associated fatty liver disease (MAFLD). *Opuntia stricta* var. *dillenii* (OD) and *Opuntia ficus-indica* (OFI), from the *Cactaceae* family, are rich in bioactive compounds, such as flavonoids, phenolic acids and betalains, that could help in the prevention of hepatic steatosis [1]. The aim of this study was to analyse the prevention capacity of OD peel extract and OFI pulp extract in hepatic oxidative stress and inflammation in a model of murine diet-induced liver steatosis. For this, 60 male Wistar rats were assigned into 6 groups (n = 10) and fed either a standard diet (C group) or a high-fat high-fructose diet (HFHF group), supplemented or not with 25 mg OD peel extract/kg body weight (b.w.)/day (L-OD group), 100 mg OD peel extract/kg b.w./day (H-OD group), 25 mg OFI pulp extract/kg b.w./day (L-OFI group) or 100 mg OFI pulp extract/kg b.w./day (H-OFI group), during 8 weeks. Liver triglyceride (TG) content, glutathione disulfide (GSSG), glutathione (GSH) and malondihaldehyde (MDA) were determined spectrophotometrically using commercial kits. NLR family pyrin domain containing 3 (NLRP3), caspase-1, interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) protein expression were measured by Western Blot. The results showed that HFHF diet led to an increase in final body and liver weights, along with higher hepatic triglycerides. Although a significant reduction in liver weight was not observed after the administration of the extracts, L-OFI treatment decreased significantly liver TG content (-11.3 %). The HFHF diet caused oxidative stress and the lipid peroxidation as shown by the ratio GSSG/GSH and MDA content. Both, H-OD and H-OFI groups significantly decreased these parameters, compared to HFHF group (-22.1 % and -43.1 % in the ratio GSSG/GSH and -28.7 % and -32.1 % in MDA content, respectively). Related to the inflammasome, NLRP3 and caspase-1 (p=0.1) protein expression augmented with HFHF diet and L-OD treatment was able to partially prevent NLRP3 protein expression (p=0.09) (-55.2 %) and caspase-1 protein expression (-54.5 %), compared to HFHF group. Regarding, IL-1β (p=0.1) and TNF-α pro-inflammatory cytokines, both protein expression were increased in the HFHF group and were partially decreased with H-OFI treatment (-36 % and -30.8 % (p=0.08), respectively). In conclusion, L-OFI treatment seems to be the most effective treatment in the prevention of steatosis and H-OD and H-OFI treatments for the prevention of oxidative stress in a model of murine diet-induced liver steatosis. Concerning inflammation, L-OD appears to prevent the NLRP3/caspase-1 inflammasome activation HFHF-diet induced, and H-OFI avoided the diet-induced inflammation.

**References**:

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**Keywords**: *Opuntia*, steatosis, antioxidant, inflammation.

# The azo-Povarov cycloaddition, a potential bioorthogonal reaction.

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## Abstract

Bioorthogonal chemistry was first defined in 2003 by Caroline Bertozzi as any chemical reaction that can occur in a biological environment without interfering with native biochemical processes [1]. Its applications include biomolecule's imaging, cell labeling or drug delivery. A bioorthogonal reaction has to work rapidly in physiological conditions, selectively and with good yields; which makes it difficult and necessary to develop new chemical reactions capable to work in that way [2].

Here, we present the recently published azo-Povarov reaction, a new [4 + 2] cycloaddition between *N*-carbonyl aryldiazenes and a dienophile [3]. First, cyclopentadiene was used as dienophile where $Sc(OTf)_3$ was needed as catalyst requiring absence of water or moisture. The use of a more strained dienophile as *trans*-cyclooctene made the reaction work without any catalyst and with the presence of water, but very high temperatures where necessary. Finally, with *cis*,*trans*-1,5-cyclooctadiene, the reaction was almost quantitative at room temperature and in low reaction times. Furthermore, the use of fluorescent starting aryldiazenes quenched by the azo bond and latterly activated with the addition of *cis*,*trans*-1,5-cyclooctadiene makes the azo-Povarov annulation a really interesting new potential bioorthogonal reaction (Figure 1).

**Keywords:** Bioorthogonal reaction, cycloaddition, cinnolines, fluorescence.

**Graphical abstract:**

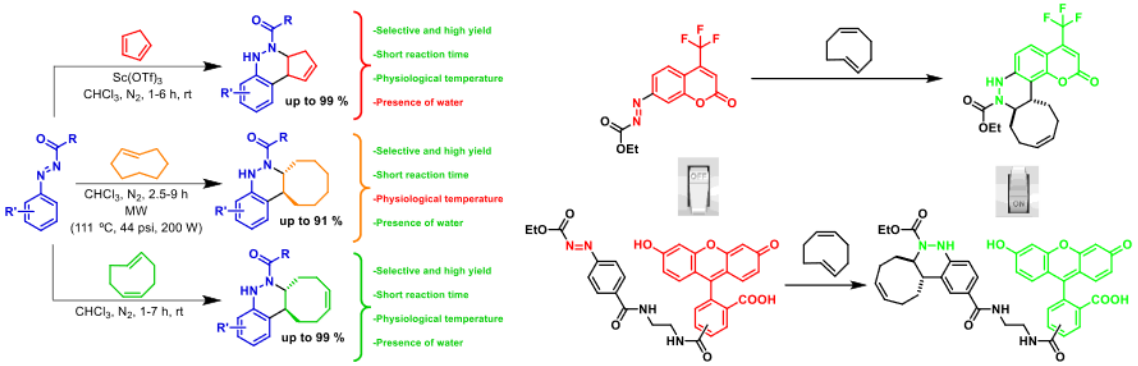


*Fig. 1.* Scope and application of the azo-Povarov reaction.

## Acknowledgments

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*I SCIENTIFIC MEETING "PASSION FOR SCIENCE", 2024*

# Phosphorated antiproliferative agents, a new alternative for cancer treatment

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**Abstract**

Cancer is a major public health concern in the world, so the search for new treatments for this disease is essential. In this way, compounds with phosphorus in their structure have proven to be effective for its treatment. As in the case of the phosphine oxide group present in Brigatinib, currently in the clinic, that is essential as a hydrogen bond acceptor that drives potency and selectivity, as well as favorable ADME properties.

On the other hand, quinolines are very important structures present in many drugs, such as antibiotics or antimicrobials.[1] They are present, for example, in Quinine and other *Cinchona* alkaloids and their derivatives, which have a lot of biological properties, including antiproliferative activity. That is the reason why our objective was to synthesize new phosphorated quinolines to evaluate their anticancer activity as Topoisomerase I inhibitors.

The well-known Povarov reaction,[3] a versatile strategy due to its chemical properties, was used for the synthesis of the phosphorated quinolines **2**, **3** and **4** (Figure 1). We started with the preparation of not commercially available phosphorated anilines **1**, and subsequent oxidation reactions, in both step by step and multicomponent procedures.

Synthesized compounds were then evaluated as antiproliferative agents in different cancerous and non-cancerous cell lines by calculating their $IC_{50}$ value and as enzymatic inhibitors with a DNA relaxation assay carried out in an electrophoresis gel, using Topoisomerase I as target.

**Keywords:** Povarov reaction, quinolines, antiproliferative activity, Topoisomerase I inhibitors.

**Graphical abstract:**

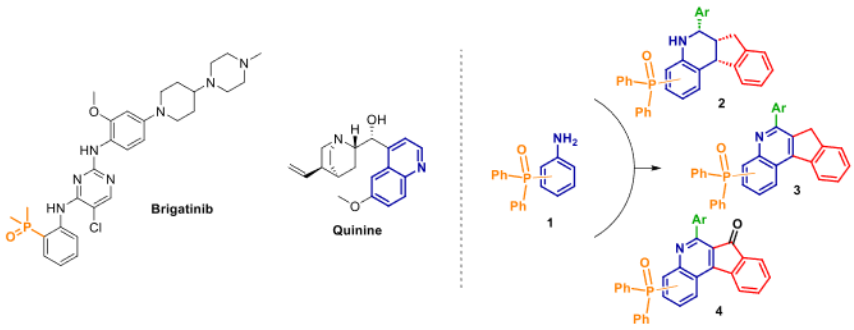


*Fig 1.* Structure of Brigatinib, Quinine and synthesized heterocycles with phosphine oxide groups.

**Acknowledgments**

Financial support PID2021-122558OB-I00 funded by the Ministerio de Ciencia, Innovación y Universidades MICIU/AEI/10.13039/501100011033 and by "ERDF A way of making Europe", and by Gobierno Vasco, Universidad del País Vasco (GV, IT1701-22; UPV/EHU) is gratefully acknowledged. The authors thank the technical and human support provided by SGIker (UPV/EHU/ERDF, EU).

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# Enantioselective synthesis of dihydroquinoline-hydantoin hybrids and their applications as antiproliferative agents.

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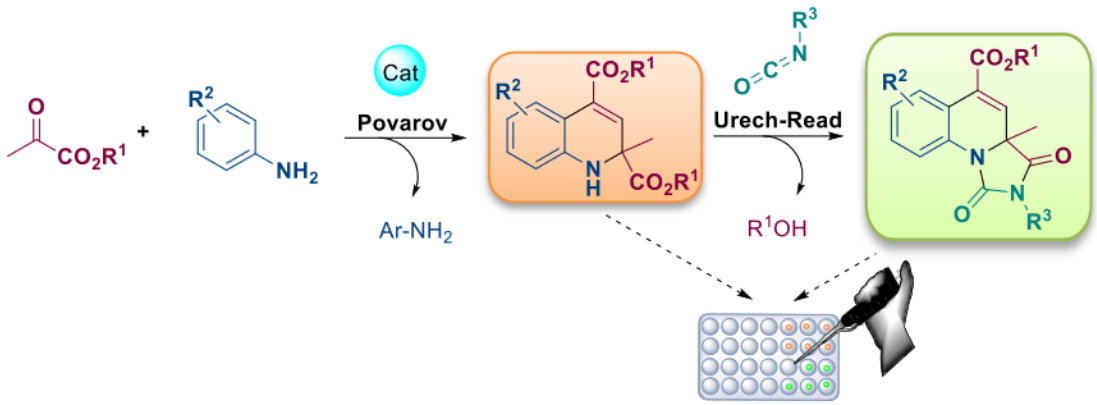
**Abstract**

Molecular hybridization is an effective strategy for designing bioactive compounds by combining different pharmacophores to create a new hybrid structure capable of interacting with multiple targets.[1]

When designing hybrid structures, *N*-Heterocyclic structures are appealing scaffolds that exhibit extraordinary pharmacological properties and are present in many biologically relevant molecules, such as nucleic acids, antibiotics, and cytotoxic agents.[2] We became interested in the combination of two different *N*-Heterocyclic bioactive scaffolds, such as dihydroquinolines and hydantoins, as a part of a hybrid system. Accordingly, herein we report an organocatalyzed enantioselective protocol involving a tandem Povarov/Urech-Read reaction, resulting in the formation of 1,2-dihydroquinoline/hydantoin hybrids through a simple "one-pot" protocol (Scheme 1). Considering the biological potential of the synthesized compounds as drugs in pharmaceutical sciences, we investigated their antiproliferative activity against various cancer cell lines, comparing the activity of the individual units with that of the hybrid structures.

**Keywords:** 1,2-dihydroquinolines, hydantoins, antiproliferative activity.

**Graphical abstract:**




*Scheme 1*. Synthesis of 1,2-dihydroquinoline/hydantoin hybrids.

**Acknowledgments**

Financial support by Ministerio de Ciencia, Innovación y Universidades (RTI 2018-101818-B-I00, MCIU/AEI/FEDER, UE), and Gobierno Vasco (GV, IT 992-16) is gratefully acknowledged. The authors thank for technical and human support provided by SGIker (UPV/EHU/ERDF, EU).

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# It is just a matter of surfaces: how carbon-based multidimensional nanocues can modulate neuronal network activity

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In the past decade, (nano)technology applications to the central nervous system (CNS) have often involved studying and using novel materials to modulate neuronal activity. The ability to govern neuronal excitability could have a significant impact not only on fundamental neurophysiology but also on developing therapeutic approaches to treat neurological diseases. In this regard, surface (nano)modification via carbon-based nanomaterials (CBNs) was demonstrated to have a pivotal role in neuromodulation. In particular, it has been shown that surfaces functionalised with carbon nanotubes [1,2] or graphene [3] are fully biocompatible and, remarkably, can induce increased network synaptic activity in interfaced neurons.
In this regard, we discovered that the neuronal network modulated could be reconducted to the synergic contribution of the nanomorphology, chemical activity, and local mechanical properties conferred by carbon nanomaterials to the surface interfacing the neuronal cells. This multimodal surface modification could be used to recapitulate the different cues the extra-cellular matrix (ECM) provides to neurons within the central nervous system. By growing neuronal cells and tissues on 2D supports and measuring the electrical activity by extra/intracellular electrodes, we demonstrated not only that neuronal activity can be modulated by exploiting different surface modifications, but also the possibility of inducing electrical reconnection in lesioned portions of the CNS.

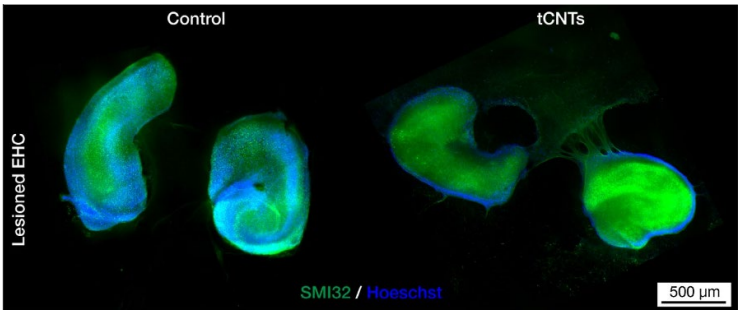


**Figure 1.** Representative images of 8-day-old lesioned entorhinal–hippocampal complex (EHC) organotypic cultures stained with Hoetsch (blue) and NeuN (green) and cultured on glass Control (left) and a CNT carpet (right). Lesioned EHC organotypic cultures display massive bundles of enthorino-dentate projections bridging the two hemisections when interfaced with CNTs, a morphological aspect associated with the reactivation of the gyrus-entorhinal (DG-EC) signal pathway.

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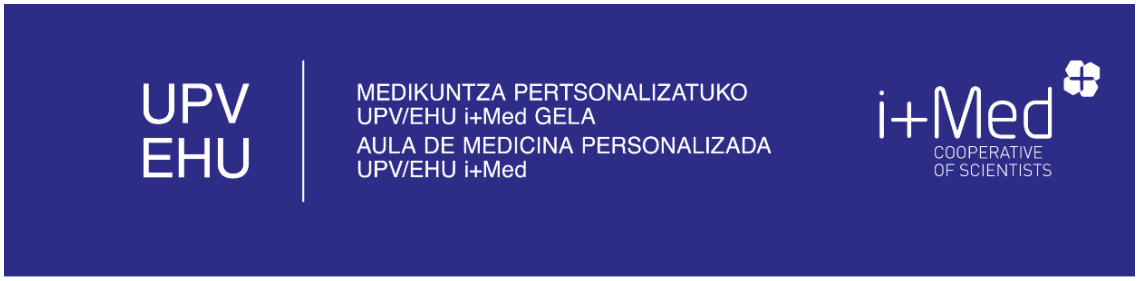
# 1st SCIENTIFIC MEETING 'PASSION FOR SCIENCE'




## POSTER COMMUNICATIONS

# Synthesis of aziridines via Joullié-Ugi reaction as potential therapeutic agents

J. Allende*, I. Olaizola, A. M. Ochoa de Retana, F. Palacios and J. M. de los Santos

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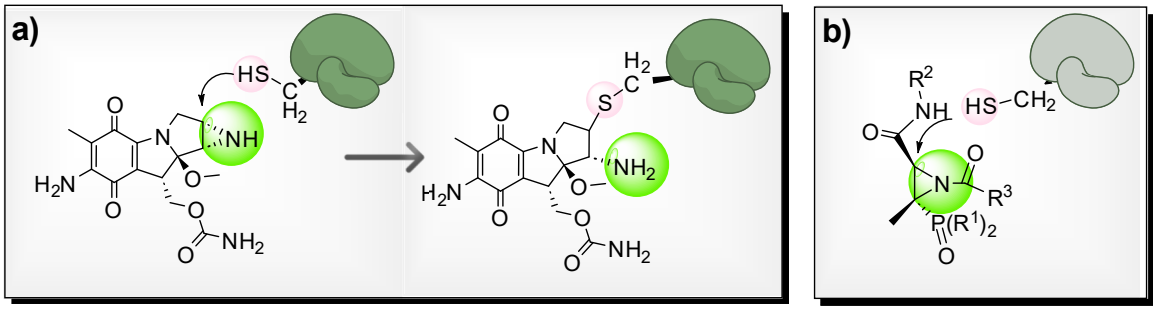
## Abstract

Cancer is a complex disease that affects one in three people. This fact makes cancer a significant public health issue, driving research into the development of new oncological drugs. Furthermore, investigations have led to new approaches in drug development, resulting in the emergence of covalent inhibitors [1]. However, the lack of effective treatments and toxicity issues persist, making the need for new drugs a primary concern. To meet this demand, organic chemists require versatile tools for drug discovery and the generation of chemical diversity. Multicomponent reactions fulfill this requirement and serve as a prototype method, allowing for the generation of complex structures from simple building blocks.

Mitomycin, a natural product, displays proven antitumor activity and is regularly used in the treatment of certain cancers. An aziridine ring remains the core structural element that initiates the action mechanism, functioning as an electrophilic motif (**scheme 1**). Considering this, our research lies in the design of new antiproliferative compounds that include an aziridine ring though the Joullié-Ugi three-component reaction. By using phosphorylated 2*H*-azirines as starting materials, the corresponding Joullié-Ugi adducts can be obtained in yields up to 85% and with very good diastereoselectivity [2]. In addition, the obtained *N*-acylaziridine derivatives can be used as synthetic precursors for the preparation 5-membered oxygen- and nitrogen-containing heterocycles, which are valuable synthons as prodrugs.

**Keywords:** *2H-Azirines, phosphorus substituted aziridines, Joullié-Ugi three-component reaction*




**Scheme 1**. a) Aziridine ring on mitomycin being attacked by a tiol residue of a protein. b) Potential action mechanism of the obtained *N*-acylaziridines.

## Acknowledgments

Financial support PID2021-122558OB-I00 funded by the Ministerio de Ciencia, Innovación y Universidades MICIU/AEI/10.13039/501100011033 and by "ERDF A way of making Europe", and by Gobierno Vasco, Universidad del País Vasco (GV, IT1701-22; UPV) is gratefully acknowledged. The authors thank the technical and human support provided by SGIker (UPV/EHU/ERDF, EU).

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# mTOR inhibition increases channel expression in a model of LQTS2 in vitro

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**Abstract**

Congenital Long QT Syndrome (LQTS) is an inherited heart disease characterized by an excessive duration of the Q-T interval. The consequence of having LQTS is an increased susceptibility to a type of arrhythmia that often degenerates into ventricular fibrillation and sudden death [1]. Approximately 40% of cases of congenital LQTS are type 2 (LQT2), i.e., caused by mutations in the KCNH2 gene, which codes for the cardiac potassium channel Kv11.1 or hERG, responsible for mediating the fast late rectifier current or IKr [2]. Current treatments are not effective in 30% of patients, therefore, it seems necessary to find new therapeutic alternatives [3].

In our research group we have demonstrated an increase in the functional expression of the hERG ion channel after treatment with a drug of the Rapalog family that inhibits mTOR (RAP). We are studying the possibility that this drug could be repositioned to form part of the therapeutic arsenal for LQT2, by restoring the expression of the hERG ion channel. The current objective is to test whether the drug RAP increases the amount of the mutated hERG channel (E58G, Y427C, R531W and L1012Pfs*55) that causes LQT2 syndrome.

Preliminary Western Blot results show that RAP increases the amount of mutated hERG channel Y427C and R531W. However, in the case of the E58G mutation no changes are observed. No immunolabeling is observed in the case of the L1012Pfs*55 mutation of the hERG channel, neither in the control situation nor after drug treatment.

**Keywords:** Congenital Long QT Syndrome type 2 (LQT2), Rapalogs, Mutated hERG Channels.

**Graphical abstract:**

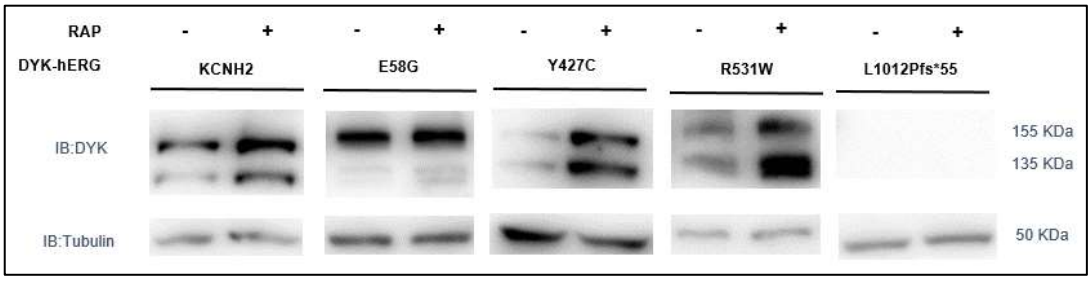


*Fig 1*. HEK-HERG cells transfected with DYK-KCNH2 or DYK-mutants, incubated with 5 uM rapa 24h. Loaded: 40 ug total protein (2 x $10^6$ cels). IB: anti-DYK (1:1000) WITH anti-Tubulin (1:1000) O/N at 4ºC. Secondary: anti-mouse (1:2000) 2h RT.

**Acknowledgments**
This work was supported by Ministry of Science and Innovation.

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# Mechanical Stimulation of Hydrogel-based Scaffolds Using a Bioreactor: Development of a Support System for Tendon Tissue Engineering

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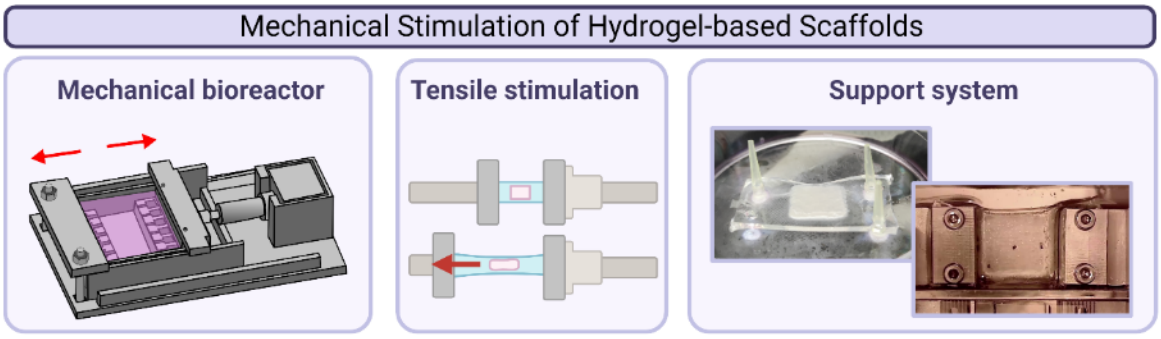
**Abstract**

Mechanical stimulation plays a crucial role in the maturation of tissue-engineered scaffolds, as it promotes cellular differentiation, extracellular matrix production, and enhances the structural and mechanical properties of the constructs [1,2]. However, one of the main challenges when using hydrogel-based scaffolds is their limited mechanical stability, which complicates their mechanical conditioning. In this study, we developed a novel system to apply mechanical stimulation to hydrogel scaffolds by utilizing a bioreactor. The system involves a support structure made from polydimethylsiloxane (PDMS), which includes a small collagen membrane. Hydrogels were printed directly onto the collagen membrane to create the scaffold. Our results demonstrate that hydrogel scaffolds were successfully printed onto the collagen membrane and remained adhered during mechanical stimulation. The scaffolds were subjected to mechanical loading conditions, with 1% strain at a frequency of 1 Hz, and remained intact throughout the process. In conclusion, we established a functional method for the mechanical stimulation of hydrogel-based scaffolds. Future work will focus on embedding cells within the scaffolds to assess cell viability, differentiation processes, and extracellular matrix production under mechanical stimulation.

**Keywords:** Mechanical stimulation, hydrogel scaffolds, bioreactor, tendon tissue engineering

**Graphical abstract:**




**Acknowledgments**

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# Influence of physical characteristics of the design of orodispersible 3D printlets on their disintegration time

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**Abstract**
3D printing allows to manufacture dosage forms with intricate designs easily (1), this can be an asset since printlet disintegration can be influenced not only by the materials constituting the dosage form, but by its 3D design as well (2). This is especially important for orodispersible tablets since they must be completely disintegrated within 3 minutes to meet the standards set by the pharmacopoeia. In our work, we analyse the influence of Sa/V ratio and total weight of various printlet designs and we have found that while total weight plays a minor role on printlet disintegration, Sa/V ratio has the most influence on the obtained results. This has been previously studied for drug release and similar results have been found (3). Lastly, since 3D printed dosage forms can display much more complex structures than those obtained through conventional methods, this manufacture method could help repurpose well known GRAS excipients with longer disintegration times for the development of orodispersible dosage forms.

**Keywords:** Printlet, orodispersible, disintegration.

**Graphical abstract:**

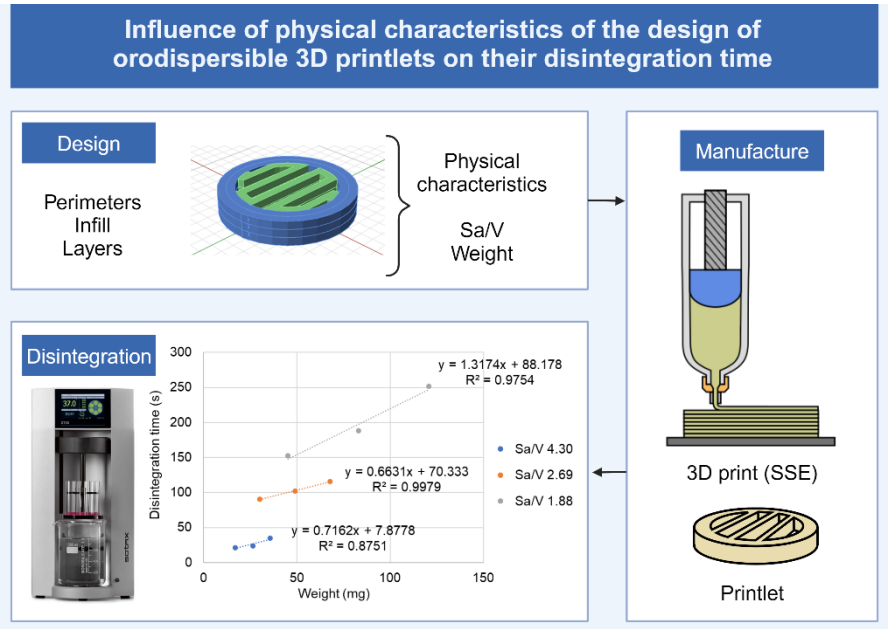


*Fig 1.* Representation of the experimental procedure and results of mean printlet disintegration times of designs with 3 recurring Sa/V ($mm^{-1}$) ratios but different weight (n=6).

**Acknowledgments**
This work was supported by the Department of Economic Development, Innovation and Demographic Challenge of the Provincial Council of Araba (Acuerdo 608/2021 del Consejo de Gobierno Foral, Araba), the Basque Country Government (Consolidated Groups, IT1448-22) and by Tecnalia Research & Innovation. Authors also wish to thank ICTS "NANBIOSIS", in particular the Drug Formulation Unit (U10) of the CIBER in Bioengineering, Biomaterials, and Nanomedicine (CIBER-BBN), at the University of the Basque Country (UPV/EHU) in Vitoria-Gasteiz.

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*I SCIENTIFIC MEETING "PASSION FOR SCIENCE", 2024*

# Development and evaluation of a bioink for the 3D bioprinting of in vitro chronic wound models

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**Abstract**

Chronic wounds negatively impact on patients' quality of life [1] and their prevalence is expected to rise. The limitations of animal models in predicting human responses to treatments [2] further complicate the situation. Therefore, there is a strong need for developing 3D bioprinted in vitro chronic wound models for testing new treatments at preclinical phases. This study presents the development of a porcine-derived decellularized extracellular matrix (dECM) bioink loaded with L929 cells to demonstrate its potential as a component for chronic wound models. The impact of different cellular loads on the rheological properties of the bioink was evaluated and compared (Fig. 1A). A LIVE/DEAD assay (Fig. 1B) and an ELISA for quantification of macrophage colony-stimulating factor (M-CSF) release (Fig. 1C) were conducted to determine the cytocompatibility and mass-transport properties of the dECM. Finally, a construct was bioprinted to assess its printability (Fig. 1D) and the impact of the selected bioprinting parameters on cell viability. After observing the pseudoplastic behavior of the bioink, the high survival rate of encapsulated cells, the release of M-CSF by them and the development of their characteristic morphology after six days, it can be stated that the bioink represents a good starting point for the development of an in vitro chronic wound model.

**Keywords:** in vitro skin model, 3D bioprinting, decellularized extracellular matrix (dECM), chronic wounds.

**Graphical abstract:**

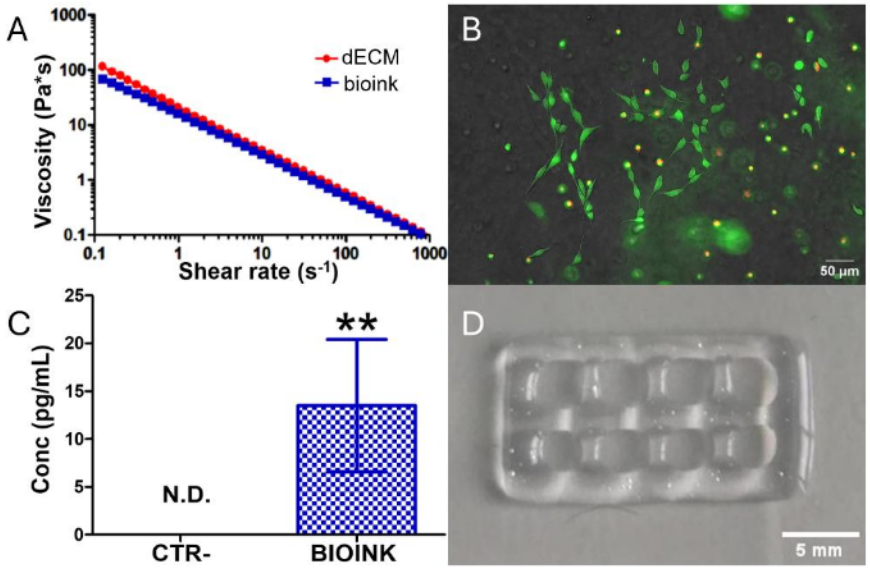


*Fig 1. A: Shear rate sweep. B: LIVE/DEAD assay of the bioink after 144 hours of incubation. C: Concentration of M-CSF present in the supernatant after 144 hours of incubation. D: Bioprinted construct.*

# Regulatory framework for 3D bioprinted corneal constructs: From design to market authorization

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**Abstract**

The regulatory pathway for 3D bioprinted corneal constructs demands alignment with the regulatory frameworks established for Advanced Therapy Medicinal Products (ATMPs). These frameworks, governed by the European Medicines Agency (EMA), require compliance with both Directive 2001/83/EC [1] and Regulation No. 726/2004/EC [2]. The design and development process must adhere to Good Manufacturing Practice (GMP) [3], ensuring product traceability and safety. Additionally, clinical trials must follow the Risk-Based Approach (RBA) guidelines for ATMPs, and products must undergo rigorous evaluation for marketing authorization through a centralized procedure. Collaboration with regulatory bodies like the Committee for Advanced Therapies (CAT), who plays a crucial role in overseeing the classification, quality, safety, and efficacy assessments of ATMPs, the Committee for Medicinal Products for Human Use (CHMP), and the Innovation Task Force is essential to meet all regulatory and safety requirements before commercialization.

**Keywords:** Advanced Therapy, Regulatory, cornea, 3D bioprinting

**Graphical abstract:**

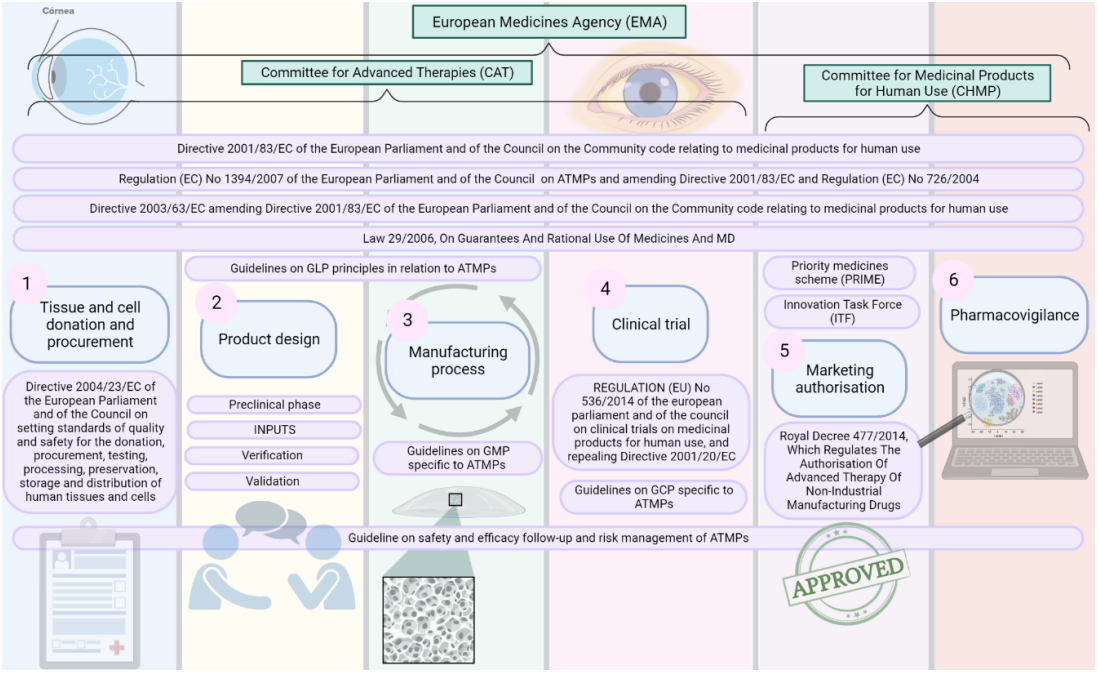


*Fig 1*. Diagram of the regulatory pathway followed by ATMPs.

**Acknowledgments**

The research has been funded by the R&D Business Support Program HAZITEK. The initiative is co-financed by the Basque Government and the European Union through the European Regional Development Fund 2021-2027 (ERDF). Project No. ZL-2021/00578 BIOINKOR – Generation of biomimetic corneal tissue as an alternative to current keratoprosthesis implantation.

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# Bioink Enrichment with Growth Factor-Loaded Nanoparticles: Enhancing Clinical Efficacy of Regenerative Scaffolds

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**Abstract**

Contemporary medical practice confronts a wide range of injuries and pathologies. In response, regenerative medicine and tissue engineering have emerged, employing scaffolds as a way to deal with these defects [1]. However, it is often necessary to enhance them with biologically active molecules, such as growth factors [2]. However, these compounds are very sensitive to different agents, both physical and chemical. Consequently, it is essential to encapsulate them in nanoparticles [3]. Moreover, due to the bioactivity of these factors at remarkably low concentrations, a sustained release over time is preferable to a fast release. Hence, the integration of a bioink, bound to nanoparticles loaded with growth factors, presents a potential strategy for enhancing of the scaffolds. This approach shows promise for improving the clinical treatment of injuries and pathologies.

In the present study, the changes in the mechanical properties of the bioink upon the addition of nanoparticles were examined. Furthermore, the release profile of the encapsulated growth factors was investigated.

**Keywords:** Nanoparticles, release profile, bioink, 3D printing

**Graphical abstract:**

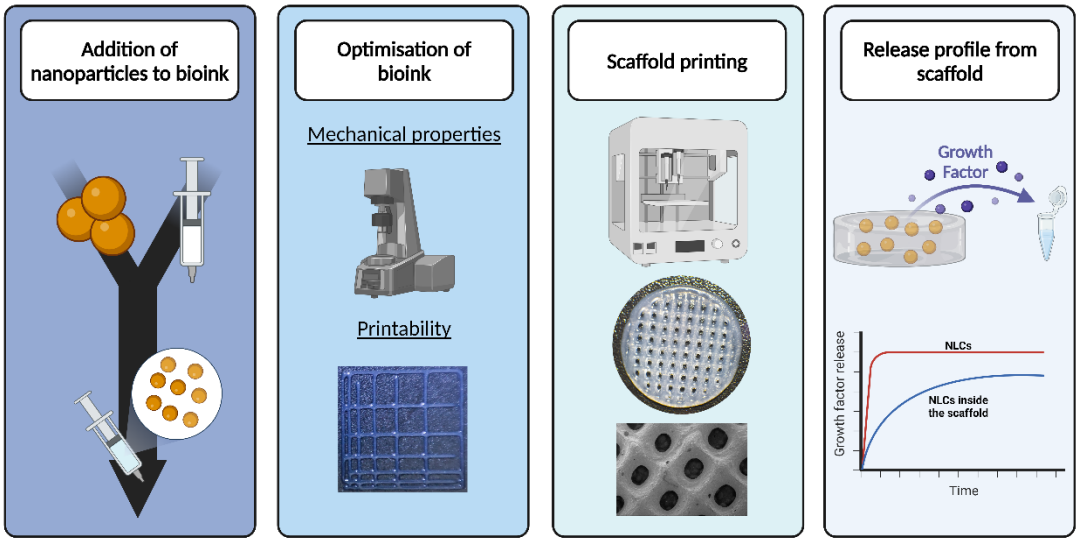


*Fig 1*. Summary schematic of the work carried out in the abstract, showing the addition of the nanoparticles, the optimization of the bioink, the scaffold printing process and its release profile.

**Acknowledgments**

This project was supported by the Basque Country Government (Grant IT1448-22), and by the TriaAnkle European project (Horizon 2020, TRIankle 952981-2). This research was also supported by CIBER (CB06/01/1028, ISCIII, Ministerio de Ciencia e Innovación). Authors wish to thank the intellectual and technical assistance from the ICTS "NANBIOSIS," U10 (drug formulation) of the CIBERBBN at the University of the Basque Country (UPV/EHU). The authors thank for technical and human support provided by SGIker (UPV/EHU/ERDF, EU).

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# Addition of *Gamma*-tocopherol rich Extract: impact on sunflower oil degradation process at frying temperatures

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It is well known that during frying, oils degrade and give rise to potentially toxic compounds that could migrate to fried food and be absorbed through the diet. In order to preserve oil quality and safety, compounds showing antioxidant capacity are frequently added, such as tocopherols. Nevertheless, most studies investigating their effect are conducted at room temperature or under accelerated storage conditions, which may not accurately reflect their performance at high temperatures, due to the complexity of the frying process. Furthermore, studies carried out under frying temperatures often employ classical methodologies, which offer limited information.

To shed light on this topic, the present study addressed the impact of adding a natural Tocopherols Extract rich in *gamma*-isoform at 0.5 % and 3 % on sunflower oil degradation process under frying conditions in the absence of food, until the legal limit of 25 % of Total Polar Compounds (TPC) was reached. Oil composition was periodically analyzed by Proton Nuclear Magnetic Resonance ($^1$H NMR) spectroscopy and the evolution of viscosity and % TPC in the oil were also assessed.

The results of this study evidenced that the higher the enrichment degree of the oil, the higher the degradation of linoleic acyl groups and the lower the formation of *E,E*-2,4-alkadienals, *E*-2 alkenals, alkanals and 4-oxoalkanals. On the contrary, at the highest addition level, the generation of *Z,E*-2,4-alkadienals and of mono-keto-*Z,E* and *E,E*-octadecadienoates was promoted. Regarding polymerization reactions, these were enhanced in the presence of the extract, considering oil viscosity values. Finally, both enriched oils reached the legal limit of 25 % TPC before the non-enriched oil.

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# DEVELOPMENT OF HYDROGEN SUFLIDE-ACTIVATED THERANOSTIC PRODRUGS FOR SELECTIVE CANCER TREATMENT THROUGH TETRAZINE DYNAMIC CHEMISTRY

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**Abstract**

A novel approach for the synthesis of hydrogen sulphide ($H_2S$) activated theragnostic prodrugs is the dynamic nucleophilic aromatic substitution of tetrazines ($S_NTz$). The dynamic covalent chemistry (DCC) of tetrazines provides a reversible and environmentally sensitive system, ideal for targeted release of therapeutic products. Therefore, it becomes a targeted administration in cancer therapy with the objective of mitigating adverse effects and improving the efficiency of the drugs. [1] Overexpression of $H_2S$ in cancer cells, such as colon cancer, triggers the release of the therapeutic agent camptothecin (CPT), a naturally topoisomerase inhibitor known for its instability, which this system aims to stabilise, along with a fluorescent marker.[2] This dual release mechanism not only promises greater selectivity towards malignant cells, but also allows the compound to be tracked, marking an important step forward in theragnostic therapy applications.[3] It is worth highlighting the potential of tetrazine-based systems in the development of sensitive and selective therapeutic solutions, particularly for conditions marked by elevated $H_2S$ levels. This strategy opens up to incorporate a variety of cytotoxic agents and luminescent probes, expanding the scope for innovative modalities to detect and treat cancer and other $H_2S$-related diseases.

**Keywords:** Cancer, Tetrazine, Topoisomerase, Camptothecin

**Graphical abstract:**

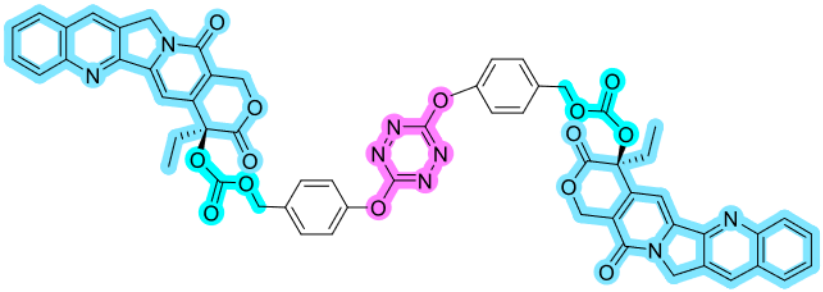

*Figure 1.* Chemical structure of self-inmolative system based on tetrazine.

**Acknowledgments**

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# Simple synthesis of 1,2-dihydroquinoline/hydantoin hybrids and the evaluation of their anticancer properties

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**Abstract**

The concept of polypharmacology refers to the ability of a medication to act simultaneously in different therapeutic contexts. This feature allows a drug, either to attack a single disease in multiple ways or several different diseases in unison, thus increasing the effectiveness of treatments [1]. Within this field, the hybrid molecule approach, where two functional groups are combined into a single structure that is capable of modulating different types of therapeutic targets, has become increasingly important during the last years [2].

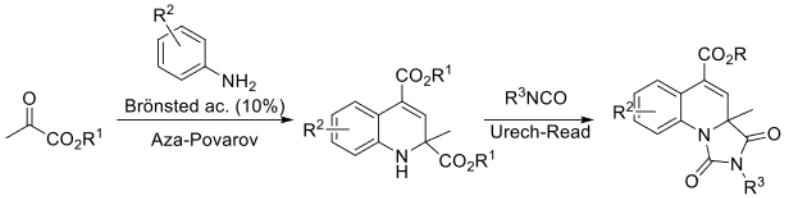
In this context, nitrogen-containing heterocycles are undoubtedly one of the most extensive families of organic compounds, widely used in many areas of the life sciences [3]. In particular, hydantoins and quinolines are two well known scaffolds that can be found in the structure of numerous natural products and drugs [4].

In view of the importance of both kinds of heterocycles, herein a simple method for the preparation of dihydroquinoline/hydantoin hybrids is described. The protocol consists of a an initial Brønsted acid catalyzed aza-Povarov reaction leading to a 1,2-dihydroqinoline intermediate, and a subsequent tandem Urech-Read reaction with an isocyanate reagent that allows the insertion of an hydantoin core in an efficient "one pot" procedure.

Willing to put into manifest the importance of the hybrid molecule approach into the pharmaceutical industry, the *in vitro* cytotoxicity of dihydroquinoline/hydantoin hybrids was evaluated towards lung (A549) and ovarian (SKOV3) human cancer cell lines. The biological activity of the hybrids was compared to that of single dihydroquinoline and hydantoin units, leading us to the conclusion that the activity of the hybrid molecules is enhanced if compared to the individual units that form the hybrid structure.

**Keywords:** Hydantoines, 1,2-dihydroquinolines, hybrid molecules, cancer.

**Graphical abstract:**




*Synthesis of 1,2-dihydroquinoline/hydantoin hybrids*

**Acknowledgments**

Financial support by Ministerio de Ciencia, Innovación y Universidades (RTI2018-101818-B-I00, MCIU/AEI/FEDER, UE), and Gobierno Vasco (GV, IT 992-16) is gratefully acknowledged. The authors thank for technical and human support provided by SGIker (UPV/EHU/ERDF, EU). A. Pellicer thank the University of the Basque Country UPV/EHU for a predoctoral grant.

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# Determination of the MBIC and MBEC of amikacin, aztreonam and meropenem in *Pseudomonas aeruginosa* biofilms

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**Abstract**

The eradication of *Pseudomonas aeruginosa* from lung infections is hampered by the bacterium´s ability to form biofilms. Biofilms limit the action of antibiotics by conferring a long-term persistence capacity [1]. The objective was to determine the minimum inhibitory concentration (MIC) and the minimum inhibitory and bactericidal biofilm concentrations (MBIC and MBEC) in 24 isolates from respiratory samples from the University Hospital of Alava against amikacin, aztreonam and meropenem [2, 3, 4].

A minimum increase of one dilution was observed between the MIC value and the CMIB and CMEB values. The latter values were markedly elevated for aztreonam, exceeding the biofilm eradication concentration of 4096 µg/mL in a considerable number of the isolates, in some cases up to a concentration of 25000 µg/mL.

In the strains studied, conventional treatments for *P. aeruginosa* infections are not very effective. The obtained MBEC and MBIC of the antimicrobials tested are not viable for use in patients. It is therefore imperative to identify effective therapeutic alternatives as soon as possible.

**Keywords:** *Pseudomonas aeruginosa*, biofilms, antimicrobial resistance

**Graphical abstract:**

***Table 1*: results of MIC, MBIC, MBEC with aztreonam.**

| Strain | MIC | MBIC | MBEC | Strain | MIC | MBIC | MBEC |
|---|---|---|---|---|---|---|---|
| PA- 22 | 0,25 | 1024 | 4096 | PA- 74 | 0,25 | 256 | 2048 |
| PA- 25 | 0,25 | 128 | 1024 | PA- 75 | 0.5 | 4 | 512 |
| PA- 33 | 0,25 | 512 | 2048 | PA-118 | 4 | 12500 | 12500 |
| PA- 35 | 0,25 | 16 | 2048 | PA-132 | 2 | 2048 | 2048 |
| PA- 40 | 0,5 | 2 | 512 | PA-138 | 8 | 4096 | 4096 |
| PA-49 | 4 | 4096 | 4096 | PA-141 | 1 | 12500 | 25000 |
| PA-52 | 2 | 2 | 4096 | PA-142 | 4 | 25000 | 25000 |
| PA- 65 | 0,25 | 256 | 1024 | PA-146 | 2 | 6250 | 25000 |
| PA- 67 | 0,25 | 16 | 512 | PA-157 | 4 | 2048 | 4096 |
| PA- 70 | 0,25 | 2 | 64 | PA-162 | 4 | 12500 | 12500 |

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# Gene therapy for dry age-related macular degeneration with ionizable nanovectors: mRNA vs pDNA

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**Abstract**

Dry age-related macular degeneration (dAMD) is a degenerative retinal disease that produces a deterioration of the retinal pigment epithelium (RPE), resulting in blurred vision and irreversible blindness. The dysregulation of the complement cascade exhibits a robust relationship with dAMD. Complement factor H (CFH) downregulates the alternative complement cascade by preventing C3 convertase formation, offering a target for specific treatment of dAMD. Most clinical trials with CFH activators have failed to demonstrate efficacy due to inability to diffuse or permeate through the vitreous and retina [1]. The aim of this work is the development of a retinal gene therapy strategy against dAMD by inducing the endogenous CFH expression. To achieve this goal, nanodelivery systems based on solid lipid nanoparticles (SLNs) for intravitreal administration of nucleic acids have been designed and evaluated in retinal cells. SLNs containing Precirol ATO®, Tween 80, the cationic lipid DOTAP and the ionizable lipids MC3 or DODAP, were prepared by hot melt (HM) and solvent evaporation (EE) emulsification methods. The influence of the lipid composition, acidification of the SLN suspension (4,5 vs 7) and the combination with different ligands were evaluated. Final vectors contained CleanCap™ EGFP mRNA or pcDNA3-EGFP, protamine, a polysaccharide, hyaluronic acid (HA) or dextran (DX), and gold nanoparticles (GN) [2]. Nanoformulations presented optimal physicochemical characteristic for ocular administration (100-300 nm, PDI<0.4 and positive superficial charge) and capacity to bind, protect, and release pDNA and mRNA. Nanovectors with GNs prepared at acid pH showed a lower particle size and a higher protection capacity. In human Retinal Pigment Epithelial (ARPE-19) cells, MC3-containing nanovectors were more effective than those containing DODAP. In addition, the protein expression kinetics was influenced by both the nucleic acid and the ionizable lipid. mRNA formulations showed the highest transfection efficacy at 48 h. The maximum transfection for pDNA formulations was at 72 h, except for SLNs containing MC3, which provided faster GFP expression, with a peak at 24 h. Nanovectors prepared with GNs increased transfection efficacy and transmission electron microscopy studies allowed us to locate the GNs into the cytoplasm of the ARPE-19 cells. The highest percent of transfected cells (80%) was achieved with vectors delivering mRNA, formulated with HA and GNs. In conclusion, nanovectors designed showed a high transfection efficacy for gene supplementation and suitable features for ocular administration, paving the way for a further in vivo evaluation as retinal delivery systems.

**Keywords:** ionizable nanovectors; pDNA; mRNA; retina.

**Graphical abstract:**

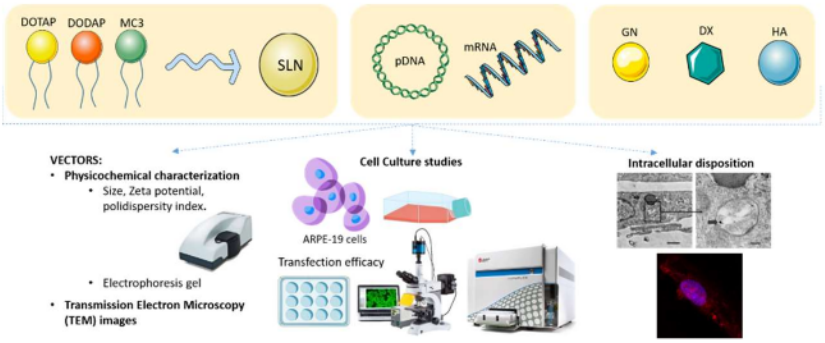


*Fig 1*. Formulation and characterization of nanovectors and cell culture studies in ARPE-19 cells.

**Acknowledgments**

P. Fernández-Muro and M. Arribas-Galarreta thank the University of the Basque Country UPV/EHU (PIFG23/45) and the Basque Government (PRE_2022_1_0144) for their research grants. This work was founded the MCIU/AEI/FEDER, UE (PID2022-139800OB-I00) and by Basque Government (IT1587-22).



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# SYNTHESIS OF NITROGEN HETEROCYLES AS HUMAN TOPOISOMERASE I INHIBITORS

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**Keywords:** *cancer, topoisomerase I inhibitors, heterocycles, camptothecin.*

Over the years, a great effort has been made to discover and develop new anticancer drugs. Among the drugs used in chemotherapy we can find the called topoisomerase inhibitors, which, currently are used in therapies that have topoisomerase I (Top I) and II (Top II) as their pharmacological target. In fact, the effectiveness of this type of inhibitors lies in the overexpression of Top I in cancer cells. Among the natural anticancer drugs targeting Top I, the most representative example is camptothecin (CPT)[2], however, the antitumor activity of CPT decreases rapidly at physiological conditions (pH: 7.4, 37ºC)[3].

In this work, a new methodology is reported for the synthesis of new polyheterocyclic compounds *via* an efficient tandem cycloaddition reaction, where the reaction between bromopyridines with halogenated or alkyl substituents and aminoalcohols or aldehydes yields pyridopyrimidine derivatives **I**, **III** and pyridoquinazoline derivatives **II** (Figure 1).[1]

In addition, the biological activity of the new heterocycles **I**, **II** and **III** was tested, and as a result, some of them showed good inhibition against human Top I enzyme and high cytotoxicity against different cancerous cell lines.

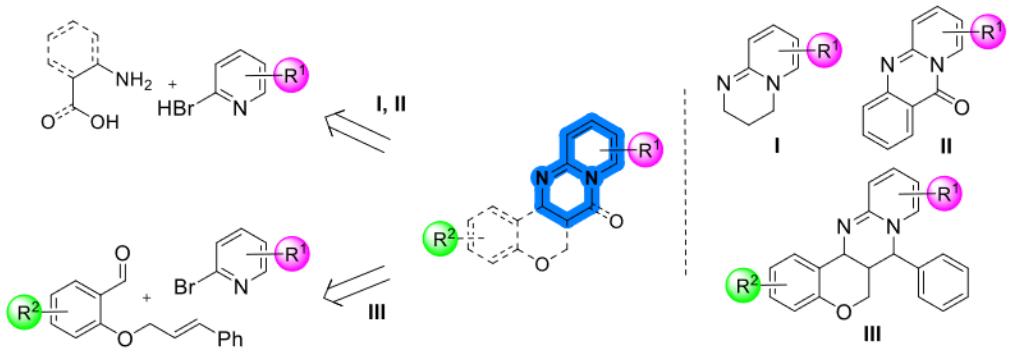


**Figure 1.** Synthesis of new polyheterocyclic derivatives **I**, **II** and **III**.

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# Towards alternative diagnostics tools for fungal infections in patients with Cystic Fibrosis

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**Abstract**

Cystic fibrosis (CF) is the most frequent genetic hereditary human disease among Caucasian people. This disease is a consequence of mutations in the CF Transmembrane Regulator (CFTR). The most serious consequence is the production of a thick and sticky mucus in the respiratory tract, enabling the entrapment of microorganisms that will colonize the airways and may cause infections [1]. Among filamentous fungi, *Scedosporium/Lomentospora* are the second most prevalent in patients with CF (pwCF), only after *Aspergillus*. The culture of respiratory samples is the technique used for their diagnosis, despite its low sensitivity, time-consuming nature and the qualified personnel required. Our research group has developed an ELISA platform using fungal crude extracts that identifies pwCF with *Scedosporium/Lomentospora*, and discriminates them from those with *Aspergillus* [2]. This technique was later transferred to a Dot Immunobinding Assay (DIA) portable system that allows obtaining the result in less than 15 minutes, which facilitates its use as a Point Of Care Testing (POCT) system [3].

Our research is also focused on the exploration of new diagnostic targets to avoid the cross-reactivity that can occur when using crude extracts. For that, immunoproteomics-based studies have been carried out, which have allowed the identification of 22 specific *Scedosporium* antigens, recognized by sera from pwCF with *Scedosporium/Lomentospora*. Some of these antigens were detected with an elevated seroprevalence, and we are currently producing some of them as recombinant proteins. Furthermore, we have studied the interest of one of the most important compounds of *Scedosporium/Lomentospora* cell wall*:* peptidorhamnomannans (PRM). For that, PRMs were extracted, purified, and their reactivity against sera from pwCF was analyzed by ELISA. We have determined that PRMs enabled discrimination between groups of pwCF with and without *Scedosporium/Lomentospora*. Thus, the PRMs seem to be an interesting diagnostic target, although its utility should be further analyzed using a larger number of sera.

**Keywords:** antigens; *Scedosporium/Lomentospora;* Cystic Fibrosis; immunoproteomic

**Graphical abstract:**

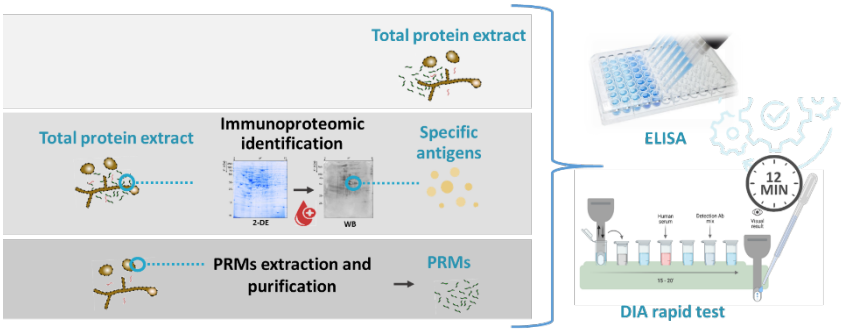


*Fig 1*. Workflow for the development of new diagnostic techniques.

**Acknowledgments**

This work was supported by the Basque Government (IT1657-22).

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# The importance of health care in the preferred-language chosen by the patient from a quality perspective

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**Abstract**

The conceptual definition of quality of health care used by the World Health Organization (WHO) is an important reference with a systemic approach [1]. Providing care must responds to individual preferences, needs and values of patient. Moreover, the use of local language and shared understanding are essential roles of quality in the delivery of health care services [2]. In Osakidetza (Basque Health Service) more than 300,000 users have requested health care in Euskara (Basque). Our objective is to spread the hypothesis among health professionals and service users that oral and written comprehensive quality health care should be provided in the preferred-language chosen by the patient [3]. To accomplish this goal our first step has been the research carried out in 2023 with medical students of the UPV/EHU, by which we have analyzed the perception of health care services and its relationship with quality of health care through a specific questionnaire based on previous research carried out in Wales (GIEB-UPV/EHU 2023/3/23; M10/2023/104). In this work we have collected information to adapt the questionnaire to our context. In resume, those who have answered the questionnaire in Euskara have described easier, more relaxed and more comfortable communication in Euskara with the doctor, and closer and more personal attention with better quality. On the other hand, some of those who have answered the questionnaire in Spanish live the demand for knowledge of Euskara as an imposition not related with quality. Finally, in the future it would be interesting to carry out a study that follows this methodology in the different villages of Basque Country. This future study would not only provide evidences on the subject, but would also affect the knowledge and awareness of health service users and professionals.

**Keywords:** quality health care, preferred-language, Euskara.

**Graphical abstract:**

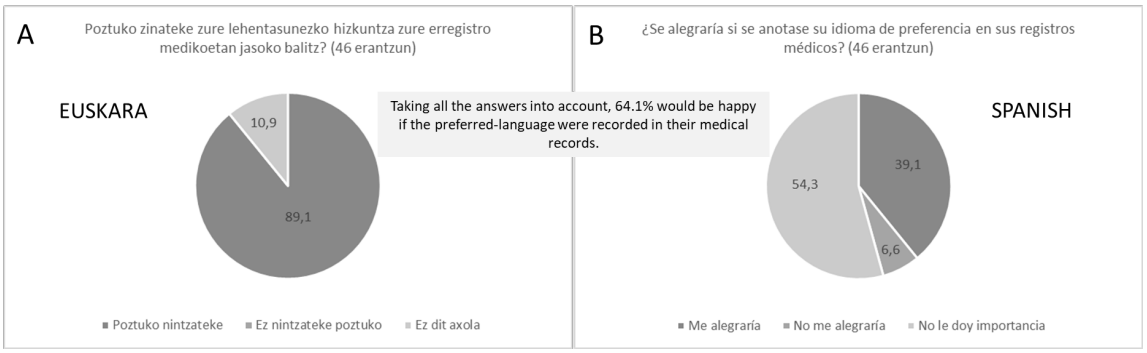


*Fig 1*. Results of the following question: Would you be happy if your preferred-language was recorded in your medical records? Those who answered in Euskara (A) and Spanish (B).

**Acknowledgments**

This work was supported by the collaboration of the Faculty of Medicine and Nursing from the UPV/EHU and Dr. Morgan research group from the University of Swansea. Thank you to the students who answered the questionnaire.

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# Potential anti-obesity effects of three different microalgae extracts

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## Abstract

Microalgae have attracted great interest in recent years due to their potential value in future sustainable, cosmetic, energy and food industries, among others. In relation to the latter, microalgae can be consumed as functional foods or used to make supplements (1). It is known that microalgae are a good source of proteins and bioactive compounds, such as polyphenols and carotenoids, which have been shown to have several health beneficial effects, such as anti-obesity (2-4). The main objective of this research was to analyse the effects of four doses of three microalgae extracts on lipid accumulation in 3T3-L1 pre-adipocytes and mature adipocytes. The selected microalgae were *Tetradesmus obliquus, Phaeodactilum tricornutum* and *Desmodesmus armatus*. One gram of freeze-dried microalgae was sonicated in a mixture of ethanol (100%) and distilled water at 70:30 (v/v) to break the microalgae cell wall. Next, the samples were kept in an orbital shaker at 4ºC overnight, then centrifuged to remove the pellet of microalgae residue and ethanol was evaporated under $N_2$ steam. Finally, the extracts were freeze-dried. For cell treatments, the selected doses, 6.25, 12.5, 25 and 50 µg/mL were tested in 3T3-L1 pre-adipocytes during the 8 days of the differentiation process and in mature adipocytes for 24h, on day 12 after the differentiation induction. The viability of the treated cells was determined by staining live cells with crystal violet (5). To analyse the effect of the extract on lipid accumulation, the amount of triglycerides was determined using a commercial kit (Spinreact, Girona, Spain), and the protein content was determined by the Bradford method (6). While in mature adipocytes none of the treatments induced significant reductions, in maturing pre-adipocytes some microalgae extracts exerted a delipidating effect. These preliminary results suggest that the tested microalgae extracts have a higher impact in the adipogenic process of maturing pre-adipocytes than in lipid metabolism of mature adipocytes.

**Keywords:** microalgae; obesity; polyphenols; carotenoids.

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# Antimicrobial and biosurfactant production by halophilic bacteria

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**Abstract**

Halophilic microorganisms, thriving in extreme environments, produce valuable compounds like antimicrobials and biosurfactants, making them promise for industrial, pharmaceutical, and environmental applications [1]. The objective of this research is to investigate the antimicrobial and biosurfactant production capacity of a collection of halophilic bacterial isolates, utilizing glycerol and glucose as carbon sources. Additionally, the study aims to assess the ability of these isolates to utilize waste materials, such as used cooking oil (UCO) and diesel, as growth substrates. The production of antimicrobials was analyzed using antagonism and diffusion assays. The production of biosurfactants was evaluated by emulsion assays (E24 index) along with the Oil Spreading (OS) and Drop Collapse (DC) tests. The study identified 20 isolates producing antimicrobials. Additionally, 14 isolates using glucose and 24 using glycerol were identified as effective producers of biosurfactants. Thirty isolates grew using UCO and 22 isolates using diesel as carbon sources. Based on the results obtained, complementary studies will be undertaken to expand upon these findings.

**Keywords:** antimicrobials, biosurfactants, halophiles, waste materials.

**Graphical abstract:**

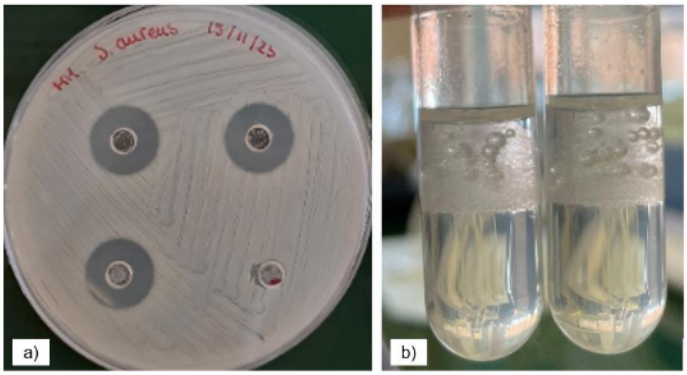


*Fig 1. Examples of the results of the conducted assays: a) diffusion assay illustrating bacterial growth inhibition by antimicrobials produced by the tested isolates; b) emulsification assay demonstrating the ability of the tested isolates to generate stable emulsions by means of biosurfactant production.*

**Acknowledgments**

This work was supported by UPV/EHU (GIU 21/021), Basque Government (PIBA_2023_1_0036) and the Añana Salt Valley Foundation.

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# 3D bioprinting of a novel graphene-based (bio)ink for tissue engineering

Irene Diez-Aldama[1,2,3,5], Fatima Garcia-Villen[4], L Saenz-del-Burgo[1,2,3], Denis Scaini[1,2,3,5,6], Jose Luis Pedraz [1,2,3,5]

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**Abstract**

Different collagen (COL) and graphene oxide (GO) concentrations and preparation protocols have been tested to guarantee optimal characteristics of the final bioink. Specifically, the bioink and the 3D scaffolds obtained via extrusion-based 3D bioprinting have been evaluated in their rheological and mechanical properties, respectively. We demonstrated that the rheological properties of the GO+COL bioink are improved while presenting similar printability. On the other hand, the compression module of the scaffolds prepared with the modified bioink is higher. The constructs have also been characterised in their micro and macro morphology using scanning electron and optical microscopies, appearing comparable in both aspects. Furthermore, to verify that the new formulation containing collagen and graphene oxide was not toxic to cells, comparative cytotoxicity, viability, and cell proliferation assays were conducted using a mouse fibroblast cell line (L-929). As proof of the principle of the use of these bioinks for vascular applications, we 3D bioprinted a portion of a vascular conduit wall by exploiting two different cell types: a mouse myoblast cell line (C2C12) and an endothelial cell line (primary human umbilical vein endothelial cells, HUVEC). Cells have been live-stained with two different cell trackers following cell development, morphology, and migration within the construct.

**Keywords:** Graphene Oxide, bioink, 3D bioprinting, tissue engineering.

**Graphical abstract:**

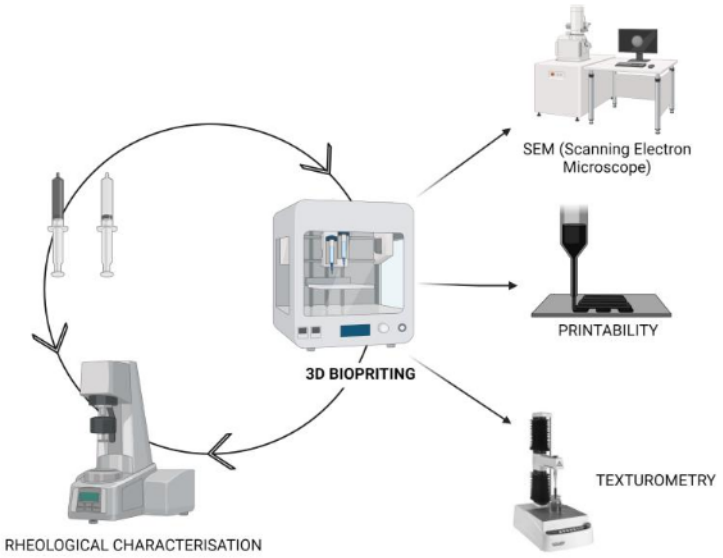


**Fig 1.** Outline of the methods used to characterise the different bio-inks.

# Investigating T cell activation through cytokine secretion using a novel cell monitoring platform

Naiara Lartitegui-Meneses[1,†], Enrique Azuaje-Hualde[1,†], Sara Lpz. de Lacalle-Arregui[1], Fernando Benito-Lopez[1] and Lourdes Basabe-Desmonts[1,2]

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**Abstract**

T cell activation occurs when the T cell receptor (TCR) binds to an antigenic peptide presented by the major histocompatibility complex, triggering cytoskeletal changes, gene expression, and the secretion of cytokines like IL-2 and IFN-γ [1]. Other co-receptors of T cells, such as CD3 and CD4, also participate in T cell activation, though their specific functions are not fully understood. To study the roll of each co-receptor in T cell activation, in this work we used the CellStudio platform [2] to generate controlled microenvironments for capturing and activating Jurkat cells, a model of T cells, and we detected their IFN-γ secretion as a marker of T cell activation. Four activation surfaces (P1-P4) were examined with varying combinations of fibronectin and anti-CD3/anti-CD4 antibodies. The results demonstrate that CD3 plays a crucial role in T cell activation, resulting in elevated secretion of IFN-γ upon activation, while CD4 alone has minimal impact on secretion (see Figure 1 C). However, the combination of CD3 and CD4 receptor activation leads to significantly enhanced secretion of IFN-γ, making their interaction essential for optimal cellular activation. Thus, although CD4 activation alone does not have a major effect, our results point to a modulation role of CD4 in the impact of CD3-mediated activation. Finally, CellStudio emerges as a promising tool for studying cell behaviour in different preconditioned scenarios.

**Keywords:** T cell receptor, activation, secretion, cytokines

**Graphical abstract:**

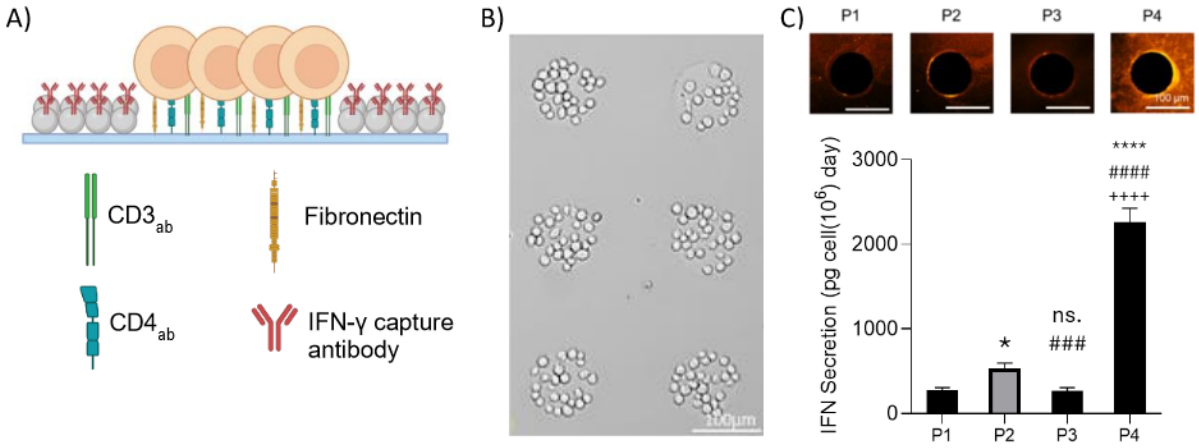


*Fig 1. A) Graphical scheme and brightfield microscope image of CellStudio platform where Jurkat cell islets are surrounded by capture antibodies of IFN-γ. B) Brightfield microscope image of Jurkat cell islets in CellStudio platform. C) Graphical representation and fluorescence images of the detected secretion of IFN-γ in each P1-P4 cases: P1 consisted of fibronectin only; P2 included a mix of fibronectin combined with $CD3_{ab}$; P3 involved fibronectin and $CD4_{ab}$; and P4 was composed of fibronectin, $CD3_{ab}$ and $CD4_{ab}$.*

**Acknowledgments**

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*I SCIENTIFIC MEETING "PASSION FOR SCIENCE", 2024*

# Real-time visualisation of biochemical streptavidin-biotin interaction using gold nanoparticles and fiber optic

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**Abstract**

The basis for many diagnostic assays, which require the formation of a specific irreversible bond between biological macromolecules, is the high affinity of the non-covalent biological interaction between biotin and streptavidin. This high affinity is the result of factors such as the formation of multiple hydrogen bonds and van der Waals interactions, together with the ordering of the surface polypeptide loops that bury biotin within the protein. [1, 2] The gold standard technique to measure and monitor such type of interactions is Surface Plasmon Resonance (SPR). SPR instruments are costly and therefore are not highly available. [3] In this work we show a novel low cost set up to measure non-covalent biological interactions. It consists on an optical systems comprised of multimode optical fibers, which tips are chemically modified to specifically interact with coated gold nanoparticles. The binding of the Au-Np to the surface of the optical fiber results in a characteristic plasmonic signal. The use of gold nanoparticles (Au-NP) to design sensors are expanding widely due to their plasmonic properties. [4] Our set up is a small benchtop set up, portable, with a cost below 2000€ and a high sensitivity, down to 3000 Au-Np per $\mu m^2$. [5] Herein, we studied the interaction streptavidin – biotin with the optical fiber set up. Specifically, we used a competitive assay of streptavidin coated 40 nm gold nanoparticles (Au-Np), which bound to biotin moieties getting immobilized on the tip of the optical fiber in the presence of increasing concentrations of free streptavidin in solution. By increasing the concentration of free streptavidin in the solution a decrease of the plasmon signal was observed, in agreement with a lower amount of Au-Np captured at the surface of the fiber. Once the model was created and tested, it is intended to be applied for the detection of β-lactam antibiotics.

**Keywords:** optical fiber, gold nanoparticles, surface plasmon resonance.

**Acknowledgments**

This work was supported by “Ministerio de Ciencia y Educación de España” under grant PID2020-120313GB-I00 / AIE / 10.13039/501100011033 and acknowledge funding support from Basque Government, under Grupos Consolidados with Grant No. IT1633-22, and ELKARTEK 23/67 KK-2023/00070. Also thank the MCIU/AEI/FEDER PID2021-122558OB-100, EU, GV/EJ (IT-1701-22) for financial support.

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# Alginate/$TiO_2$ Bead Biosystem for Cholesterol Determination in a Microfluidic device

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**Abstract**

Cholesterol monitoring is essential for diagnosing and managing cardiovascular diseases, motivating the development of efficient, rapid, and cost-effective biosensing platforms for near patient use. This paper reports an alginate/ titanium dioxide ($TiO_2$)-based biosystem for cholesterol determination. Alginate/$TiO_2$ beads with integrated enzymatic mix were calibrated and verified with cholesterol concentrations 1 - 8 mM (physiological range [1]), setting the detection time at 8 min. As shown in previous work [2], the integration of $TiO_2$ nanotubes improved the detection capabilities of the biosystem due to its superhydrophilic character [3]. Moreover, the $TiO_2$ nanotubes provide to the alginate scaffold with an intense white coloration that improve colorimetric detection, in contrast to the transparent color of traditional alginate beads. The biosystem was then integrated into a microfluidic platform as a proof of concept, allowing multiple analysis of the sample along the channel, demonstrating the sensing capabilities that this material will provide to the microfluidic field.

**Keywords:** cholesterol detection, alginate bead biosystem, microfluidic device

**Graphical abstract:**

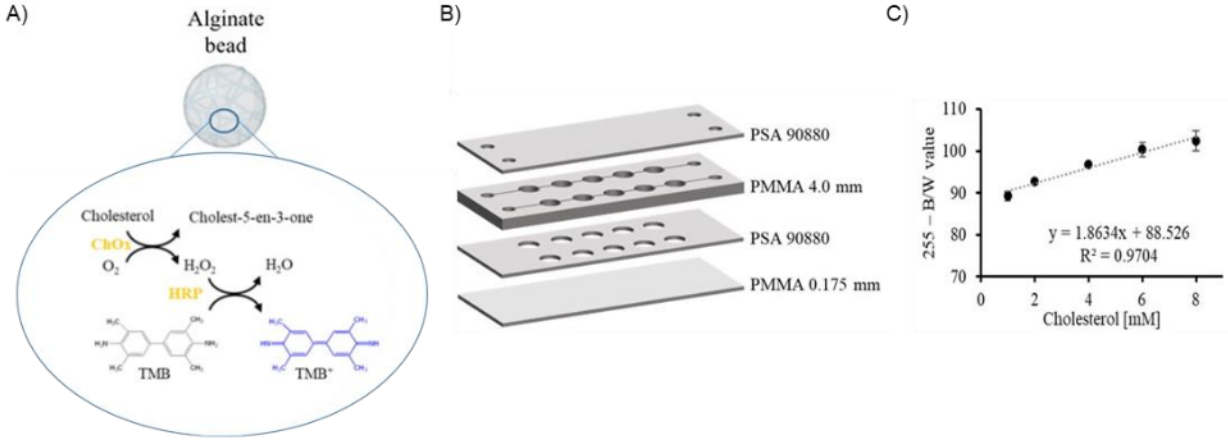


*Fig 1*. Alginate/$TiO_2$ bead biosystem for cholesterol determination in a microfluidic device. A) Scheme of the colorimetric assay consisting of cholesterol oxidase (ChOx), horseradish peroxidase (HRP) and 3,3',5,5'-Tetramethylbenzidine (TMB). B) Representation of the layers of the microfluidic device with two independent channels for cholesterol sensing. C) Calibration curve for cholesterol 1 – 8 mM in alginate/$TiO_2$ beads at 9 min (n = 3).

**Acknowledgments**

This work was supported by the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 778001 (DNASurf), the European Union's Horizon 2020 research and innovation program under grant agreement No. 766007, the "Ministerio de Ciencia y Educación de España" under grant PID2020-120313GB-I00 / AIE / 10.13039/501100011033 and the Basque Government (Grant IT1633-22).

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# Synthesis of functionalized chitosan polymeric nanoparticles and their properties for controlled drug release

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**Abstract**

Chitosan is a natural polymer whose biomedical use is being widely studied due to its excellent bioactive properties[1]. In addition, work is also being done to extend biomedical capabilities through modifications with functional groups. Synthesis of ammonio-quaternized chitosan derivatives by functionalization through chemical reactions and purification processes have been done to obtain different chitosan nanoparticles (fCS-NP) with differential characteristics, such as greater mucoadhesion to biological tissues[2]. Their ultimate purpose will be their utility as carriers for active ingredients with controlled release capacity[3], in this case, aimed at improvements in eye care treatments. Chitosan amino quaternary derivatives with substitution percentages between 20% and 30% can be obtained by controlling the amount of reagent added. Nanoparticles of about 100 – 180 nm are obtained, both empty and loaded with active principles and of pure chitosan or its derivatives.

**Keywords:** chitosan, nanoparticle, mucoadhesion, drug release.

**Graphical abstract:**

Parameters

| Response | Goal | Lower | Target | Upper | Weight | Importance |
|---|---|---|---|---|---|---|
| pdi | Minimum | | 0,11 | 0,82 | 1 | 1 |
| SIZE | Minimum | | 104,88 | 465,17 | 1 | 1 |

Solution

| Solution | pH | %Sust | PM | pdi Fit | SIZE Fit | Composite Desirability |
|---|---|---|---|---|---|---|
| 1 | 5,8 | 30 | HMW | 0,175 | 133,19 | 0,914915 |

*Fig 1*: fCS-NPs synthesis optimization

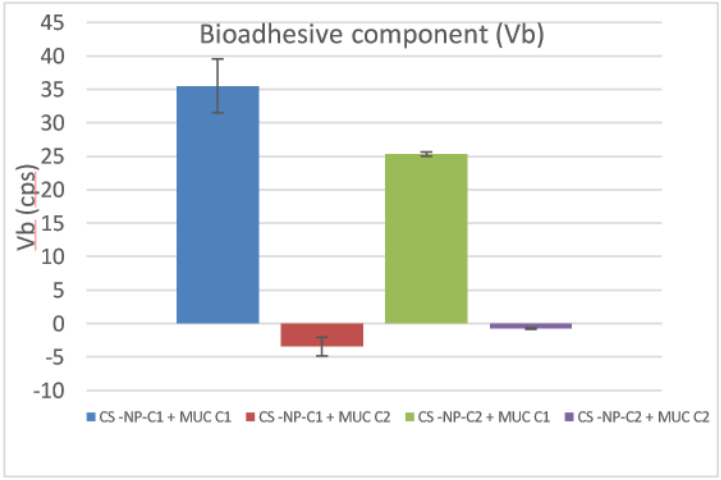


*Fig 2:* Bioadhesive component of fCS-NP + mucin mixtures

**Acknowledgments**

This work has been made possible thanks to the resources and support provided by I+Med, S.coop. and the *Programa Bikaintek 2022- expedient number 011-B2/2022* and has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 871650, MEDIPOL project.

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# Showcasing the potential of different pharmacokinetic modeling approaches for the optimization of antimicrobial therapy

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**Abstract**

Antimicrobial resistance represents one of the most pressing challenges facing global health today [1]. Different pharmacometric procedures, such as population pharmacokinetics (PopPK), physiologically based pharmacokinetic (PBPK) modeling, and PK/pharmacodynamic (PD) analysis with Monte Carlo simulations, are crucial for optimizing drug therapy [2]. The objective of this study was to demonstrate the applicability of different pharmacokinetic modeling approaches for the evaluation and optimization of dosage regimens of widely prescribed antibiotics in different population groups. The antibiotics evaluated included piperacillin/tazobactam, meropenem and ceftaroline, used to treat serious infections in critically ill patients [3], and fosfomycin, widely used for treating women's uncomplicated urinary tract infections, which are among the most prevalent infections worldwide [4]. The different models developed in the study allowed us to characterize the PK profile of the drugs evaluated and to predict their efficacy in different populations considering the main microorganisms responsible for the infections.

**Keywords:** antibiotics, population pharmacokinetics, pharmacokinetic/pharmacodynamic analysis, physiologically based pharmacokinetic model.

**Graphical abstract:**

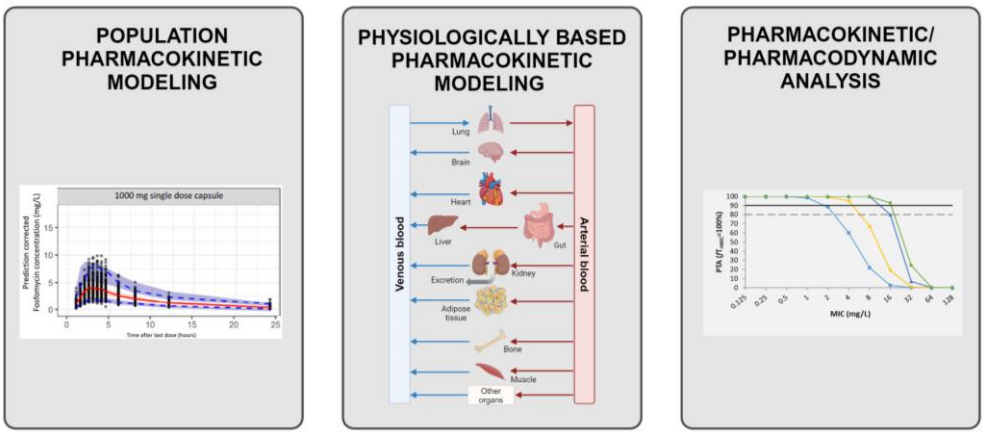


*Fig 1*. Different pharmacokinetic modeling approaches used in this study.

**Acknowledgments**

The authors acknowledge the Basque Government for the financial support (EOM2021078, IT1587-22, 2021111049, PIBA 2019-57).

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*I SCIENTIFIC MEETING "PASSION FOR SCIENCE", 2024*

# *DUBs substrates: deciphering the UCH family*

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**Abstract**

Deubiquitinating enzymes (DUBs) are involved in the regulation for a plethora of physiological processes by removing ubiquitin moieties from a wide variety of substrates [1]. The human genome encodes over 100 DUBs and it remains a mystery what are the substrates of each DUB and what is the overlapping, if there is any, between distinct DUBs [2]. Based on mechanistic and structural features, DUBs are classified into seven families: UCH, USP, OUT, MJD, MINDY, ZUP and JMM. In the present study, we aimed to unveil the substrates of the UCH family [3], which is comprised of four members (UCHL1, UCHL3, UCHL5 and BAP1).

**Keywords:** ubiquitin, substrates, DUB, bioUb

**Graphical abstract:**

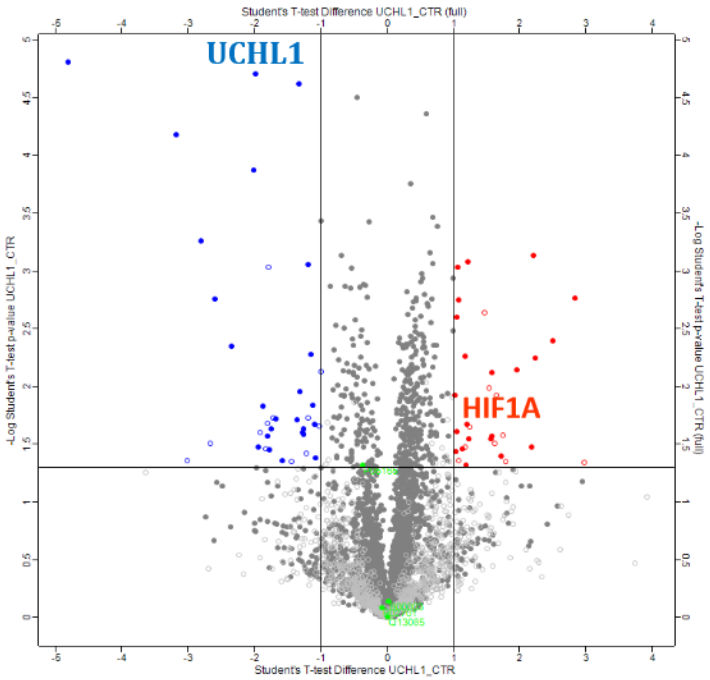


*Figure 2.* Putative UCHL1 substrates. Volcano plot showing differentially ubiquitinated proteins between UCHL1 silencing and control human cell lines (HEK cells).

**Acknowledgments**

This work was supported by Dr. Kerman Aloria, Proteomics Core Facility Specialist at the Advanced Research Facilities (SGIker), UPV/EHU.

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# Advances in Cell Affinity Assays with SCADA: Simple and Rapid Analysis of Cell-Molecule Interactions.

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## Abstract

The interaction between tumor cells and their microenvironment is crucial for tumor progression and metastasis. Measuring the affinity between cancer cells and molecules immobilized on solid substrates can advance drug discovery and the development of targeted therapies [1,2]. This study aims to optimize the characterization of cell adhesion to specific substrates using a microarray platform called SCADA (Single Cell Adhesion Dot Array) [3]. Our primary goal is to refine the technique for analyzing cell affinity, focusing on reducing the consumption of cells and molecules of interest while using accessible equipment for efficient and straightforward visualization of results. We created a microarray via UV light-assisted micropatterning that enables the digital quantification of cell adhesion, facilitating affinity assays involving interactions between cells and immobilized molecules. By comparing HeLa cells and human hair follicle-derived mesenchymal stem cells (h-HF-MSCs), the analysis revealed that fibronectin exhibited the highest cellular affinity. Furthermore, molecules such as polyethylene glycol (PEG) and bovine serum albumin (BSA) demonstrated low affinity, validating their utility as anti-adhesive agents (Figure 1). Our biosensor provides high-resolution digitalized data using a binary counting method. This platform minimizes assay time and user intervention making it compatible with simple data acquisition equipment. It serves as an efficient tool for studying cell affinity in biomedical fields, cancer research and the development of personalized treatment strategies. Ultimately, this can enhance patient outcomes and reduce healthcare costs.

**Keywords:** cell adhesion, affinity, cancer, microfluidics.

## Graphical abstract:

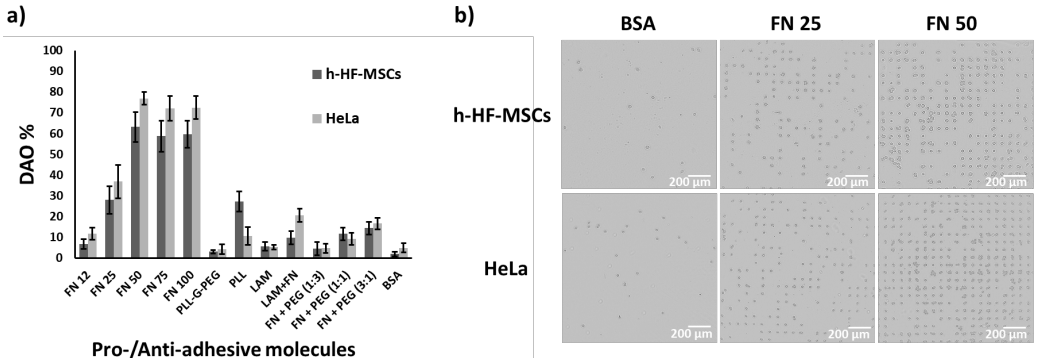


*Fig 1*. Comparison of the affinity of h-HF-MSCs and HeLa cells for different molecules: a) Graphical representation of the affinity (DAO%) to pro-/anti-adhesive molecules (FN(X)=Fibronectin (12, 25, 50, 75, 100 µg.mL$^{-1}$); PLL-g-PEG = Poly-L-Lysine-graft-polyethylene glycol; PLL= Poly-L-Lysine; LAM= Laminin; PEG= Polyethylene glycol; BSA= Bovine Serum Albumin). Error bars SDV (n = 9) b) Brightfield images of patterns coated with BSA, FN 25 µg.mL-1 and FN 50 µg.mL$^{-1}$ for both cell types.

## Acknowledgments

This work was supported by Research project MICROFLUIDICS & BIOMICS Cluster UPV/EHU (IT1633-22) financed by Basque Government.

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# Development of an Alginate Bead Biosystem for the Visual Detection of Cortisol

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**Abstract**

Cortisol plays a crucial role in the correct day-to-day behaviour of the different bodily processes [1]. therefore, the early detection of anomalies on corticosteroids concentration is of increased importance to prevent or pretreat underlying issues and prevent the development of chronic illnesses like Addisson's sickness or Cushing's syndrome. Since urine is the most common biofluid used for this analyte's determination [2], there is high interest for alternative quantification methods that can reliably measure cortisol levels on non-intrusive biofluids [3]. This research investigates the potential of an alginate based material as matrix for quick and cost-effective cortisol detection by image analysis. First, the capabilities of nitro blue tetrazolium chloride as a chromophore for detecting low concentrations of cortisol was tested, as well as the viability of an image based analysis method for quantification. Secondly, the reagents needed for the colorimetric determination of cortisol were integrated into an alginate bead biosystem capable of quantitatively measuring cortisol levels in concentrations as low as 54.96 µg $dL^{-1}$, through the previously developed image analysis method. This biosystem could, in the near future, reduce both patient and laboratory analysis derived costs for preliminary testing, since it shows promise as a point of care tool for cortisol level diagnosis.

**Keywords:** microfluidic device, bead, colorimetric reaction, cortisol

**Graphical abstract:**

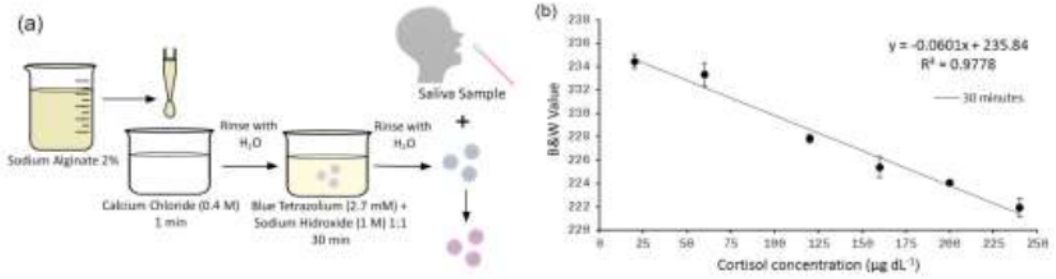


*Figure 1. (a) Scheme of the proposed bead biosystem fabrication method and it use. (b) Alginate bead calibration curve of cortisol.*

**Acknowledgments**

The authors acknowledge funding support from the MCIU under grant PID2020-120313GB-I00/AIE/ 10.13039/501100011033, FEDER, Gobierno Vasco Dpto. Educación for the consolidation of the research groups (IT1633-22), Proyecto de Investigación Fundamental Colaborativa – Investigación Fundamental ELKARTEK: KK- 2023/00070 and the Basque Government (Grant IT1633-22).

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# Assessing Breast Cancer in vitro through Smart Bioelectronics

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## Abstract

The development of advanced *in vitro* platforms that emulate *in vivo* conditions is critical for biomedical research and drug discovery, being an alternative to animal models [1]. Smart Bioelectronics, combining functional materials such as PEDOT:PSS (poly(3,4-ethylenedioxythiophene)(styrene sulfonate), bioelectronics, and microfluidics, enable precise manipulation of cellular microenvironments [2]. PEDOT:PSS, a widely utilized conductive polymer with mixed ionic and electronic conduction properties, can be biochemically and mechanically tuned to replicate specific tissues, facilitating the creation of 3D bioelectronic interfaces [3]. When integrated with microfluidic technology, these platforms allow real-time electrical and optical monitoring of cellular processes. This work presents 2D and 3D Smart Bioelectronic interfaces for the simultaneous monitoring of breast cancer cell proliferation.

**Keywords:** bioelectronics, smart materials, cancer, *in vitro* platform

**Graphical abstract:**

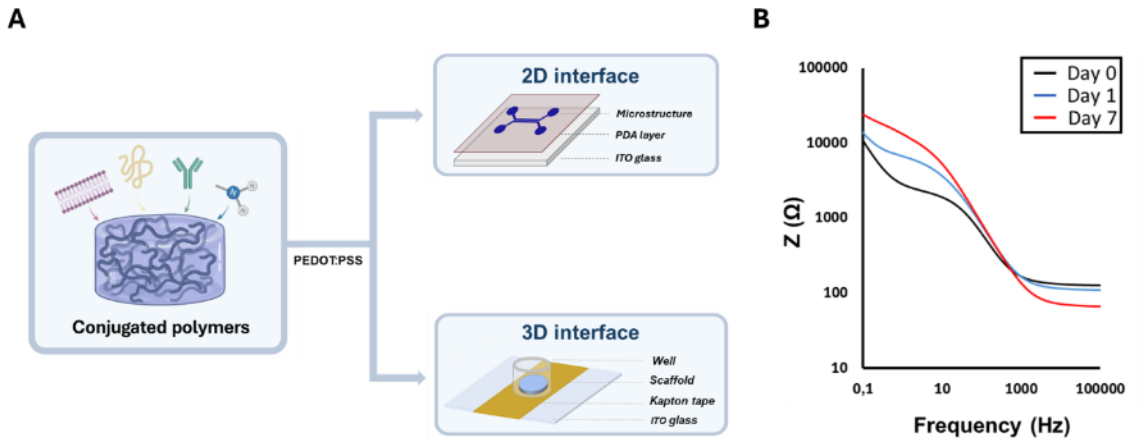


***Fig 1. 2D and 3D Interfaces using Smart Bioelectronics.*** **A.** Overview of the different types of interfaces developed in our research line. **B.** Impedance magnitude versus frequency plots for a device seeded with MDA-MB-231 cells over 7 days.

## Acknowledgments

This work was supported by the Ikerbasque, Basque Foundation for Science, Departamento de Salud del Gobierno Vasco, FUNDACION Vital Fundazioa, Gobierno de España, Ministerio de Ciencia y Educación de España" under grant PID2020-120313 GBI00/AIE/10.13039/501100011033, Departamento de Salud del Gobierno Vasco under grant 2022333036 (SAN22/10) and Gobierno Vasco Dpto. Educación for the consolidation of the research groups (IT1633-22).

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# NEW FRONTIERS IN ANTIMYCOLATA AGENT DISCOVERY: MODIFICATION OF KNOWN ANTIBIOTICS

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**Abstract**

Over the past decade, the modification of existing drugs has revolutioned the field of organic chemistry, emerging as a highly attractive strategy for medicinal chemists engaged in drug development.[1] This approach, which significantly improves the efficiency of generating new pharmacologically active derivatives, incorporates innovative strategies such as Drug Repurposing, Late-Stage Functionalization and other related methodologies. These techniques are particularly promising for the development of next-generation antibiotics, which could lead to more effective treatments capable of combating antimicrobial resistance.[2]

Despite the high potency of some antibiotics, their clinical use is diminished due to their poor absorption by the targeted microorganisms. This is particularly the case of bacteria belonging to the *Mycolata* group, which cause severe health problems such as tuberculosis, which contains an external lipophilic layer that further prevents the penetration of many drugs.

To overcome this barrier and enhance the passage of drugs through Mycolata cell membrane, our group has focused on the synthesis of increased lipophilic compounds by taking advantage of the reactivity of some of the functional groups present in the structure of the original drugs (Figure 1, 2). [3] Specifically, we chose a couple of WHO-listed essential medicines, the well-known antibiotics dapsone and sulfadoxine, which display in their structure an aniline group able to participate in multicomponent reactions.

**Keywords:** *multicomponent reactions; antibiotics; resistant bacteria; mycobacteria*

**Graphical abstract:**

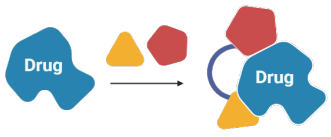


*Fig 1. Drugs from drugs* through multicomponent reactions.

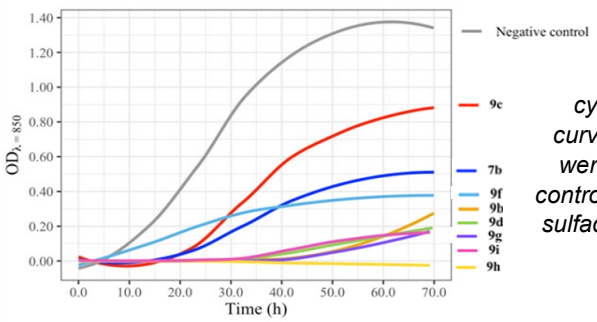


*Fig 2. Nocardia cyriacigeorgica growth curves. Several conditions were tested: the negative control (grey) and synthetized sulfadoxine derivatives at 15 g/mL.*

**Acknowledgments**

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# Effects of *Opuntia* extracts. A comparison between epididymal and subcutaneous adipose tissue in a murine model of obesity

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**Abstract**

The products derived from *Opuntia* are used in the pharmaceutical, food and cosmetic industries. Further, it has been observed that *Opuntia* extracts can have beneficial properties for the prevention and treatment of certain disorders, such as obesity and type-2 diabetes [1]. This study aimed to analyse and to compare the beneficial effects of the extracts of two different *Opuntia* species in epididymal and subcutaneous adipose tissues from rats fed an obesogenic diet. Rats were distributed into 6 groups and fed either a standard (Control) or a high-fat high-fructose diet (HFHF), supplemented or not, with *Opuntia stricta* var. *dillenii* (OD) peel extract or *Opuntia ficus-indica* var. *colorada* (OF) fruit extract, at doses of 25 (L-) or 100 (H-) mg/kg/day, for 8 weeks. Protein expression of adipose triglyceride lipase (ATGL), aquaglyceroporins (AQP7 and AQP9) and CD36 fatty acid transporter were measured by immunoblotting in both epididymal and subcutaneous tissue. *Opuntia* extracts were not able to significantly prevent the body weight increase induced by the obesogenic diet. Nevertheless, a significant reduction was observed in visceral (epididymal and mesenteric) and subcutaneous fat pad weights in the group supplemented with H-OD and L-OF extract, respectively. Concerning epididymal tissue, a trend towards reduced ATGL protein expression levels was observed in HFHF group compared to control rats ($p$=0.1). This effect was significantly prevented through H-OD and H-OF supplementations; in the case of L-OD extract a trend towards increased values was found ($p$=0.1). CD36 was up-regulated in HFHF group, in comparison with control animals. In addition, in HFHF rats AQP9, involved in glycerol uptake, was increased and AQP7, involved in glycerol release, was decreased, when compared with the control group. H-OD was able to prevent the AQP7 and AQP9 protein expression changes induced by the HFHF diet. Regarding CD36, a fatty acid transporter that under lipolytic conditions facilitates fatty acid release from adipose tissue [2], it was significantly up-regulated by both H-OD and H-OF extracts, in comparison with HFHF rats. Concerning subcutaneous tissue, ATGL protein expression was significantly decreased in HFHF group, compared to control group. This effect was markedly prevented by H-OD, L-OF and H-OF extracts. HFHF group showed a trend towards reduced AQP7 expression levels ($p$=0.06) in comparison with control group, while there were no changes in AQP9 or CD36 expression. A significant increase in CD36 expression levels was found in L-OF group, compared to rats fed only obesogenic diet. These results suggest that the obesogenic diet induces fatty acid and glycerol uptake in epididymal tissue, while this effect was not such evident in the subcutaneous tissue. Conversely, the changes induced by H-OD in the expression of ATGL, CD36 and AQPs in epididymal tissue, seem to be involved in the reduction of this tissue depot, while in the case of the subcutaneous fat pad reduction, L-OF extract seems to be the responsible for this reduction through ATGL and CD36 up-regulation.

**Keywords:** Opuntia; obesity; bioactive compounds.

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# Viable Lactobacillus rhamnosus GG shows superior prevention over its inactivated variant on high-fat high-fructose diet-induced MAFLD prevention in rats

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Metabolic-associated-fatty-liver disease (MAFLD) is characterized by an excessive fat accumulation in hepatocytes. It is associated with the consumption of westernized diets (rich in saturated fats and sugars) and to microbiota dysbiosis. The aim of this research project is to compare the potential effects of the administration of a heat-inactivated probiotic (*L. rhamnosus* GG, LGG) with the effects of the intact probiotic for the prevention of diet-induced MAFLD. 36 male Wistar rats were divided into 4 experimental groups: standard diet (C group), high-fat high-fructose diet alone (HFHF group), with the probiotic (LGG, $10^9$ CFU/day, PRO group) or with the inactivated probiotic (heat-inactivated LGG, $10^9$ CFU/day, PARA group) for 6 weeks. High-fat high-fructose feeding provoked an increase in body and hepatic weight, transaminase levels, serum and hepatic triglycerides and NAS score-associated parameters (steatosis, ballooning and inflammation). Viable LGG administration effectively reduced serum and hepatic triglyceride levels and NAS score while treatment with heat-inactivated LGG only a non-significant tendency. FATP2 expression was significantly lower in the PRO group and CD36 decreased in both experimental groups, although a sharper decrease was observed in the PARA group. FAS enzymatic activity was significantly reduced in the PARA group, while a partial reduction was observed in the PRO group. Finally, PARA group showed higher content of fecal lipids. Thus, although the administration of inactivated probiotic may reduce intestinal fat absorption (higher excretion in feces) and *de novo* lipogenesis rate, the overall lipid-reducing impact of the probiotic in the liver appears to be more significant.

# Food insecurity and health outcomes among the Spanish sub-cohort of the FINESCOP study

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## Abstract

Food insecurity (FI) has emerged as a significant public health concern, particularly among university students, a demographic often facing financial constraints and dietary challenges. The economic consequences of the COVID-19 crisis have exacerbated this issue. In addition, research indicates that FI is associated with adverse health outcomes [1], yet studies focusing on European university populations remain limited. The primary objective of this research was to assess the prevalence of FI among university students at the University of the Basque Country UPV/EHU and to explore its relationship with health outcomes. This cross-sectional study was conducted as part of the Food Insecurity among European University Students during the COVID-19 Pandemic (FINESCOP) project, which involved collaboration among multiple universities across Europe. A convenience sample of 394 students from UPV/EHU participated in the study, completing an online questionnaire that included the Food Insecurity Experience Scale (FIES) developed by the Food and Agriculture Organization (FAO) [2] and health outcomes, including body mass index (BMI), self-rated health, and overall well-being, among other data. Data collection occurred between December 2021 and January 2022. The results indicated that 19% of the surveyed students experienced some level of FI, with 2.5% reporting moderate FI and 0.8% severe FI. Notably, students facing FI exhibited significantly higher BMI and reported poorer self-rated health compared to their food-secure peers. Additionally, the study found that FI was associated with a decline in health status during the pandemic, highlighting the compounded effects of economic and health-related stressors. These findings are consistent with other studies which investigate the consequences of FI for college and university students [3,4], and suggest that FI may act as a stressor that impacts health. In conclusion, this study emphasizes the critical link between FI and health among university students, particularly in the context of the COVID-19 pandemic. The findings suggest that FI not only affects dietary intake but also has broader implications for health status. To address these challenges, higher education institutions, such as UPV/EHU, should prioritize the implementation of comprehensive food relief initiatives and supportive policies aimed at mitigating the impact of FI on student health and well-being.

**Keywords:** food insecurity, university students, health outcomes, socioeconomic status

**Acknowledgments**

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# A Novel Method for Advancing Bispecific Antibodies

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**Abstract**
Bispecific antibodies act as a molecular bridge between two different antigens, providing these molecules with novel functionalities. Encouraged by their potential clinical benefits, bispecific antibodies have entered a golden period of development. However, technical challenges associated with production yields, stability, and purity hinder the broader clinical application of these molecules. In this study, we developed a new strategy to efficiently assemble bispecific antibodies through engineering of the Fc domain. Through an iterative process of protein engineering, expression, and bispecific quantification, we have identified a condition that precludes the formation of byproducts, resulting in the formation of only properly assembled bispecific molecules. We employed several biophysical tools to demonstrate that the resulting molecules are functional, thermostable, and that the 3D organization of their Fc fragment resembles that of conventional antibodies. This strategy has the potential to advance the field of bispecifics towards biologics with more favorable manufacturing properties compared to current strategies.

**Keywords:** antibody, cancer, bispecific.

**Graphical abstract:**

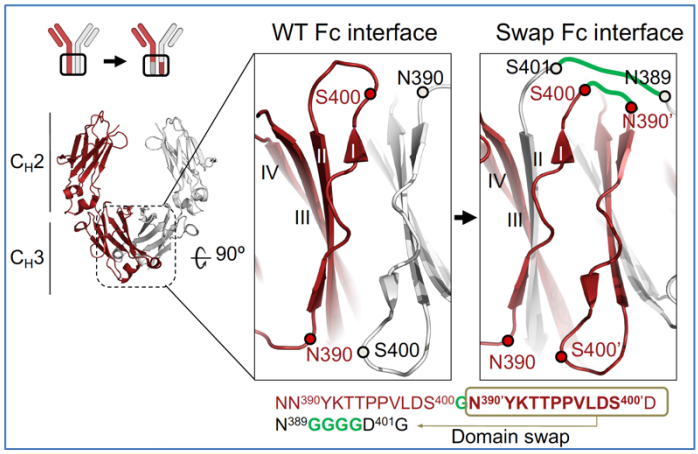


*Fig 1*. For the swap design, 11 amino acids long strand is removed from the CH3 domain of antibody-B (Ab-B) (grey) and included in the sequence of antibody-A (Ab-A, dark red). Glycine-based linkers (green) are used to connect the engineered strands. The four strands at the dimer interface are labelled (I-IV) in wild-type (WT) chain-A.

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# Functional stability of tetracycline: practical information for its use in bacterial selection

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## Abstract

Bacterial genetic manipulation has become a common methodology in many laboratories for various purposes. Antibiotic resistance genes or cassettes are frequently used as markers. Tetracycline is a commonly used antibiotic for this purpose, although its poor stability requires *in situ* preparation each time it is used. In this work, we tested the functional stability of tetracycline when stored at 4ºC for use in genetic modification experiments. A tetracycline hydrochloride stock solution (15 mg/mL) was prepared and used to supplement several BHI agar plates, which were then stored at 4°C until use. On different days, both wild-type and tetracycline-resistant mutant strains of *Escherichia coli* and *Arcobacter butzleri* were grown on BHI broth and agar supplemented with tetracycline (15 µg/mL). The MIC of all strains was also determined. All tetracycline-resistant strains were able to grow in both conditions, whereas wild-type strains were inhibited for at least 175 days after antibiotic preparation. The MIC for wild-type *E. coli* and *A. butzleri* strains was 0.25 and 8 µg/mL, respectively, while tetracycline-resistant strains had a MIC of 24 and 32 µg/mL. The tetracycline supplemented media (15 µg/mL) maintains a potency greater than 8 µg/mL for at least 175 days after preparation when stored at 4°C.This facilitates its use in genetic modification experiments without the need for in situ preparation.

**Keywords:** Tetracycline; Bacterial selection; Genetic manipulation; Antibiotic functional stability.

## Graphical abstract:

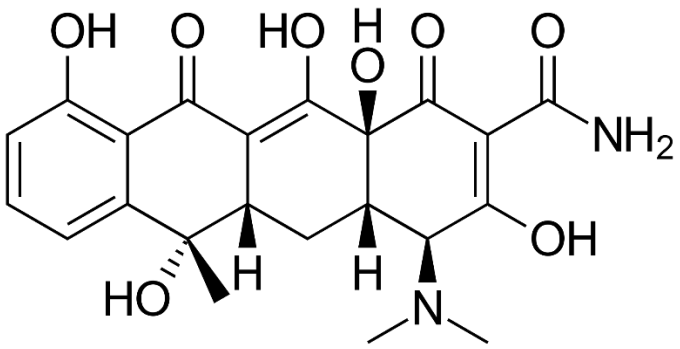


*Fig 1*. Chemical structure of the tetracycline.

## Acknowledgments

This work was supported by the project GIU21/021 (UPV/EHU). A.S.-S. has a PhD grant from the UPV/EHU.

## References

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# Construction and characterization of pIMM24, the first *Escherichia coli – Arcobacter butzleri* shuttle vector

Adrián Salazar-Sánchez[1,2,*], Rodrigo Alonso[1,2], Aurora Fernández-Astorga[1], Ilargi Martínez-Ballesteros[1,2], Lorena Laorden[1,2], Irati Martinez-Malaxetxebarria[1,2]

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## Abstract

*Arcobacter butzleri* is a foodborne human pathogen widely distributed which causes watery diarrhoea and enteritis [1]. To date, gene inactivation using suicide vectors and CRISPR-cpf1 technology were use to understand its pathogenic mechanisms [2, 3]. The aim of this work was to construct and characterise a shuttle vector capable of replicating and expressing in both *Escherichia coli* and *A. butzleri*. The pIMM24 shuttle vector was created by inserting the origin of replication of *A. butzleri* and a tetracycline resistance cassette into a cloning vector; and verified through complete sequencing. The plasmid's transformation efficiency, stability, effects in growth and tetracycline resistance were analysed. pIMM24 was validated using it to complement a non-motile *fliS*-knockout *A. butzleri* mutant [2]. The transformation efficiency of pIMM24 (7,425 bp; GenBank accession PP129559) is of 4,572 ± 2,351 CFU/µg vector in *E. coli* and of 32 ± 7 in *A. butzleri*. Without antibiotic pressure, it remains 100% stable in *E. coli* after 7 days, and 65-75% stable for 1-5 days in *A. butzleri*. The pIMM24-transformants exhibit a 4-96-fold increase in their natural MIC for tetracycline. There are no significant differences in the growths. pIMM24 restored the wild-type motility of the *fliS*-knockout mutant. In this work we present the first *E. coli – A. butzleri* shuttle vector, a valuable tool for complementation studies that contribute to the increase of knowledge of this species.

**Keywords:** *Arcobacter butzleri*; Shuttle vector, Genetic modification.

## Graphical abstract:

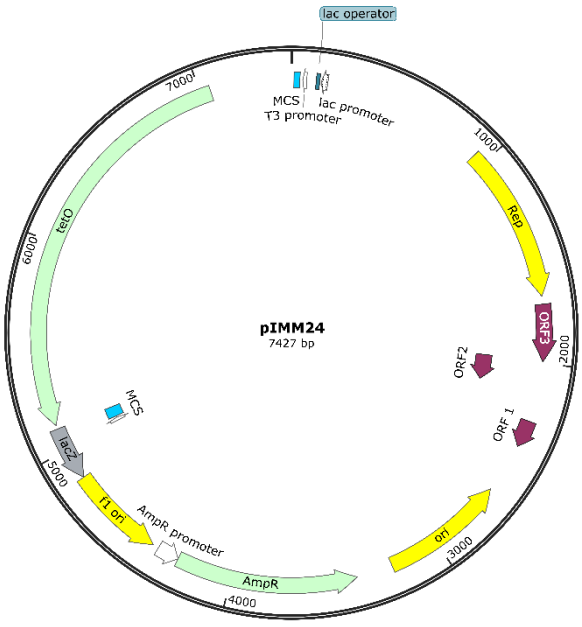


*Fig 1*. Genetic map of the plasmid vector pIMM24.

## Acknowledgments

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# 1st SCIENTIFIC MEETING 'PASSION FOR SCIENCE'

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