



Institute for Bioengineering of Catalonia

17th IBEC SYMPOSIUM

BIOENGINEERING

**FOR EMERGENT AND
ADVANCED THERAPIES**

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Welcome to IBEC's 17th annual Symposium on Bioengineering for emergent and advanced therapies!

I am happy to meet you again in our annual symposium. This Symposium is a very special one because we will celebrate our 3rd consecutive accreditation as a Severo Ochoa centre, which places IBEC among the top research centres in Spain.

I hope that you'll be stimulated and inspired by our programme of talks, posters, and networking. This year we are exploring a new format, with a special afternoon cool-off session entirely devoted to posters, combined with an outreach activity for high school students, following our endeavour to bring bioengineering to society.

Thank you very much for participating in the Symposium!

Josep Samitier

Director

Institute for Bioengineering of Catalonia (IBEC)

17th IBEC SYMPOSIUM

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Programme

Monday 21st October

16:00 – 17:00	Satellite outreach activity
17:30 – 19:30	Symposium poster session at Citilab

Tuesday 22nd October

08:00 – 09:00	Registration
09:00 – 09:15	Opening ceremony
09:15 – 09:45	<i>Director's presentation</i> Josep Samitier , Director IBEC
09:45 – 10:20	Invited Speaker: Bio fabrication and Biosensors: Monitoring and Guiding Regeneration in bone and cartilage. Valentina Basoli · <i>Biomedical Engineering Department, Basel University</i> <i>Chair: Javier Ramón · IBEC</i>
10:20 – 10:50	Thematic Networks Presentation <i>Chair: Pere Roca-Cusachs · IBEC</i>
10:50 – 11:35	Coffee break
11:35 – 12:10	Invited Speaker 2: Strategies to Promote Cancer Nanomedicine Performance and Clinical Translation Twan Lammers · <i>Aachen University</i>

12:10 – 13:10	Flash presentations. Session 1 (10 presentations) <i>Chairs: Benedetta Bolognesi i Xavier Rovira · IBEC</i>
13:10 – 13:30	Alumni session: Bridging the gap: from PhD to pharma industry Veronica Hortigüela · <i>Manufacturing Area in pharma industry, Spain</i> <i>Chair: Elena Martínez</i>
13:10 – 13:30	Group picture
13:40 – 15:00	Lunch break
15:00 – 15:25	Third Severo Ochoa Award Recognition
15:25 – 16:25	Flash presentation. Session 2 (10 presentation) <i>Chairs: Elisbete Engel i Javier Ramón · IBEC</i>
16:25 – 17:00	Invited Speaker 3: Microphysiological systems mimicking human tissue barriers for the discovery of new drug delivery strategies Tae-Eun Park · <i>Department of Biomedical Engineering at UNIST</i> <i>Chair: Samuel Sánchez · IBEC</i>
17:00 – 17:10	<i>PhD Committee</i> Inés Macías · <i>Smart Nano-Bio-Devices · IBEC</i>
17:10 – 17:20	<i>Postdoc Committee</i> Guillermo Martínez · <i>Integrative Cell and Tissue Dynamics · IBEC</i>
17:20 – 17:30	Awards and closing ceremony





Keynote Lectures

Bio fabrication and Biosensors: Monitoring and Guiding Regeneration in bone and cartilage.

Valentina Basoli

This seminar will present new advancements and technologies in tissue engineering for osteochondral regeneration, with a focus on key topics such as donor variation, advanced 3D printing techniques for cartilage and bone repair, and the increasing role of biosensors in monitoring cellular behavior, all aimed at translating these innovations to patient care. Osteochondral defects, which affect both cartilage and the underlying bone, pose a significant challenge in regenerative medicine due to the complexity of these tissues and their limited natural healing ability.

A major challenge in this field is donor variability, where cells from different donors can behave differently due to factors like age, health, and genetic background, affecting their ability to form functional tissue. This variability complicates the development of consistent regenerative treatments. The seminar will examine how these differences impact tissue engineering outcomes and discuss protocols designed to overcome these challenges.

Beyond cellular issues, the seminar will highlight the potential of 3D printing in osteochondral regeneration, utilizing various materials and specialized 3D printing platforms. The complexity of the additive manufacturing process, including technologies like melt electro-writing and hydrogels, enables the creation of anatomically precise microstructures. Additive manufacturing has revolutionized tissue engineering by allowing the creation of highly customized, patient-specific scaffolds that closely replicate the structure and function of native bone and cartilage.

Additionally, the seminar will explore the role of biosensors in tissue engineering. Both electrochemical and optical biosensors are emerging as vital tools in regenerative medicine, enabling real-time monitoring of the cellular environment within tissue scaffolds. These sensors provide continuous feedback on crucial physiological parameters such as pH, oxygen levels, and nutrient availability, which are essential for cell health and tissue growth. By integrating these sensors into engineered tissues, researchers gain real-time insights into cell behavior, enabling timely adjustments to ensure optimal tissue regeneration or model pathological conditions. This level of monitoring could significantly enhance the success of tissue-engineered implants by allowing for dynamic, responsive interventions tailored to the cells' immediate needs.



Valentina Basoli, Biomedical Engineering Department, Basel University

Valentina Basoli is a Senior Research Scientist with the Swiss Medical Additive Manufacturing Group and lecturer at the Department of Biomedical Engineering, University of Basel. Her expertise lies in biofabrication techniques, regenerative medicine, and biosensors.

She holds a Bachelor's in Molecular Biology and a Master's in Applied and Experimental Biology from the University of Sassari, Italy. Her Master's thesis was completed at the Robinson Institute for Epigenetics, University of Adelaide, Australia. Valentina then pursued a joint Ph.D. at the University of Natural Resources and Life Sciences (BOKU), Vienna, focusing on cellular responses to physical stimuli.

Her postdoctoral research at the AO Research Institute Davos included studies on mesenchymal stem cell markers, donor variability in chondrogenic differentiation, and the development of biosensors for cell quality control. She also explored mRNA transfection and nano-materials for drug delivery.

Valentina is visiting scientist at iCEMS, University of Kyoto, Japan, through the JSPS Postdoctoral Fellowship. She furthered her education with an MAS in Translational Medicine and Biomedical Entrepreneurship at SITEM, University of Bern, honing skills for translating scientific insights into practical applications and managing biotech ventures.

Valentina is the author and co-author of numerous articles in scientific journals and book chapters. Her achievements include securing national and international funding such as Innosuisse grants, Spark Swiss National Science Foundation, and EU-Pathfinder grants, EU-Horizon. She is a management committee member in the EU for the CostAction for COST CA21110, and coordinator for the Short-term scientific missions (STSM) fostering global collaboration in OsteoArthritis research.

Strategies to Promote Cancer Nanomedicine Performance and Clinical Translation

Twan Lammers

Nanomedicines are extensively explored for cancer therapy. By delivering drug molecules more effectively and more selectively to pathological sites, and by attenuating their accumulation in healthy organs and tissues, nanomedicines assist in improving the balance between (chemo)therapy efficacy and toxicity. The tumor accumulation of nanomedicines is traditionally ascribed to the EPR effect, which is highly variable, both in animal models and in patients. To address issues associated with tumor targeting heterogeneity, and to advance cancer nanomedicine clinical translation, we are working on systems and strategies to monitor and modulate tumor-targeted drug delivery. In the present lecture, several of these strategies will be highlighted, including image-guided interventions to prime tumor blood vessels and the microenvironment, as well as the use of imaging and tumor tissue biomarkers for patient stratification. These efforts help to establish rational and realistic ways forward towards promoting the clinical translation and performance of cancer nanomedicines.



Twan Lammers, Aachen University

Twan Lammers obtained a D.Sc. in Radiation Oncology from Heidelberg University in 2008 and a Ph.D. in Pharmaceutical Technology from Utrecht University in 2009. In the same year, he started the Nanomedicine and Theranostics group at RWTH Aachen University. In 2014, he was promoted to full professor of medicine at RWTH Aachen University Clinic. His group aims to individualize and improve disease treatment by combining drug targeting with imaging. To this end, image-guided (theranostic) drug delivery systems are being developed, as well as materials and methods to monitor tumor growth, angiogenesis, inflammation, fibrosis and metastasis. He has received multiple scholarships and awards, including ERC starting, consolidator and proof-of-concept grants, the CRS Young Investigator Award, the Adritelf International Award, the Belgian Society for Pharmaceutical Sciences International Award, and the JNB Trailblazer Award. He served as the president of the Controlled Release Society in 2023-2024, and he has been a council member of the European Society for Molecular Imaging for almost 10 years. He is a member of the editorial board of 10 journals, and acts as associate editor for JCR, DDTR and MIB. Since 2019, he is included in the Clarivate Analytics list of Highly Cited Researchers.

Bridging the gap: from PhD to pharma industry

Veronica Hortigüela

Technical skills developed during years of scientific research can open the door to the pharmaceutical industry. Soft skills will make a difference.

A personal example of the journey from academia to the pharma industry will be shared,

You will also get a quick glimpse of job positions that can be covered by a scientist in different pharma sectors.



Veronica Hortigüela, Manufacturing Area in pharma industry (Spain)

Verónica Hortigüela Lázaro was born in 1987 in Burgos, Spain. She finished her Degree in Biotechnology at the University of Salamanca and experienced an Erasmus year at the Katholieke University of Leuven (Belgium).

To bolster her scientific interdisciplinary skills, she studied a Master in Nanoscience and Nanotechnology at de University of Barcelona. In 2011, she started her

research activities as a master student, and later, as a PhD student at the Institute for Bioengineering of Catalonia (IBEC) focusing on cell-surface interactions at the micro&nanoscale.

Her first job in the pharmaceutical industry was in 2015 in the R&D area as Development and scale-up process specialist. In 2017, she defended her PhD thesis and was offered a new role for data analysis as Technical manager in the Manufacturing Area. From then, she has led different Manufacturing Departments and now is responsible for the Initial Process Division, leading a team of more that 180 employees towards innovation and continuous improvement.

Microphysiological systems mimicking human tissue barriers for the discovery of new drug delivery strategies

Tae-Eun Park

The Blood-Brain Barrier (BBB) is a selective barrier that controls the movement of molecules and ions between the blood and brain in the central nervous system (CNS). This makes CNS drug development challenging, as the BBB prevents foreign substances from entering the brain. To address this, researchers have focused on identifying BBB-specific ligands, with aptamers (short DNA or RNA sequences) emerging as promising candidates due to their advantages over conventional molecules. To efficiently screen aptamers, a novel MPS-SELEX technology was developed. This system mimics a human BBB using a device where brain microvascular endothelial cells (BMECs) and brain cells are cultured in separate channels. The MPS-SELEX method identified a functional BBB shuttle aptamer, hBS01, which crosses the BBB via clathrin-mediated endocytosis. hBS01 showed significantly higher BBB penetration compared to a control aptamer group and demonstrated improved organ-specific targeting in animal models. The MPS-SELEX platform also proved more effective than traditional transwell models in screening drug delivery systems. This approach holds promise for developing brain-targeted drug delivery systems and can be adapted for other organ-specific MPS models.



Tae-Eun Park, Ulsan National Institute of Science and Technology (UNIST), South Korea

Tae-Eun Park is an associate professor at Ulsan National Institute of Science and Technology (UNIST) in Korea. She obtained her PhD in 2015 from Seoul National University, developing a brain drug delivery system and during her 2015-2017 at Wyss Institute in Harvard University, she started her human microphysiological system (MPS) research. Currently, her research is concentrated on developing humanized organ mimetics using MPS and organoid technology for novel therapeutic and drug delivery solutions. Her work primarily focuses on replicating tissue barriers, understanding their cell homeostasis and cell-cell interactions. Additionally, she explores ways to effectively deliver drugs and therapeutic cells across these barriers.





FLASH Presentations

FLASH PRESENTATIONS · SESSION 1

CELL ENGINEERING

NAME	SURNAME	TITLE
Gaia	Amato	Developing Human Organoids To Model Genetic And Systemic Conditions During Congenital Anomalies Of The Kidney And Urinary Tract
Chiara	Ninfali	Duchenne Muscular Dystrophy fibro-adipogenic progenitors impair muscle function of co-cultured healthy myotubes in a functional 3D model
Karolina	Zimkowska	Monitoring Neuronal Activity in Human Cortical Organoids with Frontotemporal Lobar Degeneration-Tau (FTLD-Tau)
Ainhua	Ferret Miñana	3D bioengineered liver for the study of acute and chronic hepatic damage

MECHANOBIOLOGY

NAME	SURNAME	TITLE
Mamatha	Nijaguna	Inhibiting mechanotransduction as a novel approach for oncology therapy
Pau	Guillamat	Guidance of cellular nematics into self-shaping active surfaces
Annalisa	Calò	Mechanical phenotyping of lung cancer CAFs
Clément	Hallopeau	Mechanisms of mechanical compartmentalisation in intestinal organoids
Guillermo	Martínez Ara	An optogenetic toolset to understand and control epithelial mechanical balance
Miguel	González Martín	Designing mechanosensible molecules for the mechanical control of cellular transcription.

FLASH PRESENTATIONS · SESSION 2

ICT FOR HEALTH

NAME	SURNAME	TITLE
Gergo	Matajsz	RF Surface Coil Design for High-Throughput Metabolic Imaging using Microfluidics
Yolanda	Castillo Escario	Measuring High-Resolution Sleep Position and its Variability in Adolescents with Smartphone Accelerometers

NANOMEDICINE

NAME	SURNAME	TITLE
Shuqin	Chen	Convective Dynamics of Swarming Enzymatic Nanomotors
Núria	Blanco-Cabra	Novel Fluidic System With Controlled Share Stress For Personalized Diagnostic In Biofilm-Related Infections
Luisa	Camerin	Photoswitchable Carbamazepine Analogs for Non-Invasive Neuroinhibition <i>In Vivo</i>
Marta	Badia	A comprehensive landscape of IAPP amyloid aggregation
Cátia	D. F. Lopes	Precision nanomedicine-enabled CRISPR-powered gene therapy for efficient amyloid- β clearance across the blood-brain barrier
Antonino Nicolò	Fallica	Development of YAT2150 analogues as potent multistage antiplasmodial agents
Nina	Kostina	Harnessing nature's blueprints to design interactive synthetic cells se tiene que situar delante de Gergo Matajsz I RF Surface Coil Design for High-Throughput Metabolic Imaging using Microfluidic
Claudia	Codano	A fumarate-based nanomedicine for macrophages' phenotypic modulation

FLASH presented by:

NAME: Gaia Amato

GROUP: Pluripotency for organ regeneration

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Developing Human Organoids To Model Genetic And Systemic Conditions During Congenital Anomalies Of The Kidney And Urinary Tract

Gaia Amato¹, Carolina Tarantino¹, Daniel Moya-Rull¹, Andrés Marco Giménez¹, Elena Garreta¹, Nuria Montserrat^{1,2,3}

¹ Pluripotency for organ regeneration (PR Lab), Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain

² Networking Biomedical Research Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain

³ Catalan Institution for Research and Advanced Studies (ICREA), Spain

Congenital anomalies of the kidney and urinary tract (CAKUT) encompass a spectrum of malformations affecting the kidneys, urethra, ureters, and bladder during embryonic development. With an incidence of 4-60 per 10,000 births, CAKUT presents a significant challenge, primarily addressed through invasive treatments like urologic surgery, dialysis, or transplantation. Many of the 40 established monogenic causes of human CAKUT were initially derived as candidate genes from observations in mouse models of CAKUT and subsequently screened for their prevalence in human disease cohorts. However, the insights from mouse models, do not always directly translate to human genetics.

Human kidney organoids have emerged as crucial tools to study morphogenetic processes under healthy and disease states. When developed from human pluripotent stem cells (hPSCs), human kidney organoids represent an unprecedented tool set to study how mutations previously related to CAKUT can explain early disease phenotypes. Here we employed CRISPR/Cas9 to engineer hPSCs, creating reporter cell lines to monitor the endogenous expression of GATA3, a lineage specifier of one of the two stem progenitor cells of the kidney, namely the ureteric progenitor cells. At the same time, GATA3 represents one of the most prevalent genes leading to renal and extra-renal CAKUT manifestations in patients. Similarly, we have further generated knock-out (KO) lines in the background of the fluorescent reporter lines to investigate the impact of PAX2 and HNF1B mutations in the GATA3 lineage.

At the present time, we are assessing successful protein suppression in the KO backgrounds through Western blot and confocal microscopy analyses. Furthermore, we are validating the successful development of our CRISPR/Cas9 engineered lines through their differentiation into nephron-like kidney organoids. Importantly, we will investigate the impact of the different genetic backgrounds in extrarenal complications of CAKUT through the differentiation of these lines into cardiac and retinal organoids. The approach generated here will further allow for the investigation of maternal conditions leading to CAKUT such as gestational diabetes, hypertension, and hyperlipidemia.

FLASH presented by:

NAME: Chiara Ninfali

GROUP: Biosensors for bioengineering

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Duchenne Muscular Dystrophy fibro-adipogenic progenitors impair muscle function of co-cultured healthy myotubes in a functional 3D model

Chiara Ninfali ¹, Xiomara Fernández-Garibay ¹, Ainoa Tejedera-Villafranca ¹, Jordi Díaz-Manera ²,
Javier Ramón-Azcón ^{1,2}, Juan M. Fernández Costa ¹

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Spain

² John Walton Muscular Dystrophy Research Centre, Institute of Genetic Medicine, Newcastle University, UK

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Duchenne Muscular Dystrophy (DMD) is the most prevalent pediatric neuromuscular disease, characterized by progressive skeletal muscle breakdown due to dystrophin protein deficiency. This results in muscle fiber loss, replaced by fibrotic tissue, hindering regeneration and treatment efficacy. Fibrotic tissue originates from fibro-adipogenic progenitors (FAPs), muscle stem cells capable of fibroblast differentiation and connective tissue production. Therefore, understanding the role of FAPs in DMD is crucial for therapy development. In this work we used an innovative 3D *in vitro* model using 3D-printed casting molds to encapsulate myogenic precursors from healthy individuals together with FAPs from healthy or DMD patients. These 3D tissues are fully functional skeletal muscles, capable of contraction and response to electrical stimuli. Through this co-culture model, we analyzed the fibrotic potential of DMD FAPs and we studied its impact on muscle functionality. Muscle tissues co-encapsulated with DMD FAPs exhibited increased fibrosis and reduced contractile capacity compared to those with control FAPs. Furthermore, they exhibited lower myofiber differentiation, likely due to fibrotic impediments in fiber fusion. This model promises insights into DMD fibrotic tissue representing a new tool for the development of new antifibrotic DMD treatments.

FLASH presented by:

NAME: Karolina Zimkowska

GROUP: Molecular and cellular neurobiotechnology

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Monitoring Neuronal Activity in Human Cortical Organoids with Frontotemporal Lobar Degeneration-Tau (FTLD-Tau)

Zimkowska, Karolina ^{1,2,3,4} Riu-Villanueva, Marc ^{1,2,3,4} Oliver-De La Cruz, Jorge ⁵ Roca-Cusachs, Pere ^{5,6} Lanciego, José Luis ^{3,7} Consiglio, Antonella ⁸ del Río, José Antonio ^{1,2,3,4}

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⁸ Department of Pathology and Experimental Therapeutics, Bellvitge University Hospital-IDIBELL, Hospitalet de Llobregat, Barcelona, Spain; Institute of Biomedicine of the University of Barcelona (IBUB), Barcelona, Spain *

Microtubule-associated protein tau is crucial in neuronal health by influencing axonal transport and microtubule stabilisation. In contrast, pathological tau is associated with early cognitive decline in Alzheimer's disease (AD) and pure tauopathies, such as FTLD-tau. Research shows that endogenous tau is implicated in neuronal activity (NA), although this role of tau is poorly understood. Neuronal excitation also regulates tau by promoting extracellular release and phosphorylation. Given that chronic epilepsy animal models show prolonged tau phosphorylation, emerging research is examining the role of pathological tau in epilepsy and the mechanisms underlying it and other tauopathy comorbidities. In this respect, changes in NA in the presence of tau mutations have not yet been analysed at presymptomatic stages. Furthermore, the currently available models lack the complexity of the human brain to fully understand the underlying mechanisms of tau pathology and its effects on NA. To address this, we present an *in vitro* platform utilising cortical organoids (COs) derived from hPSCs to unravel the impact of tau pathology on NA by exploring the changes that occur after the inclusion of mutated P301L-tau or full-length non-mutated human tau (2N4R) by adeno-associated virus. We employed calcium imaging techniques using genetically encoded calcium indicators and high-speed microscopy recordings to analyse changes in NA patterns and their correlation with tau pathology. Through overexpression of P301L-tau, we successfully developed COs that exhibit hyperphosphorylated tau as observed by biochemical analysis of phospho-tau(Ser422), PHF-1, and AT8, as well as tau aggregates observed by positive staining for thioflavin-S. Additionally, we have shown that these COs have NA with

observed changes in neuron firing frequency and network connectivity. Moreover, we have found that P301L-infected COs have reduced inter-spike intervals and that the neuronal network displays reverberating super bursts relative to controls. Our findings suggest that tau mutations alter the NA of COs by producing hyperexcitable networks susceptible to seizure-like activity. Thus, we believe that tau hyperphosphorylation could be responsible for the occurrence of seizures in the early stages of certain tauopathies like FTLT-tau and even AD. This model can, therefore, be used to further explore the functional consequences of tau mutation-mediated changes of NA in tauopathies.

FLASH presented by:

NAME: Ainhoa Ferret Miñana

GROUP: Biosensors for bioengineering

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

3D bioengineered liver for the study of acute and chronic hepatic damage

Ainhoa Ferret-Miñana¹, Estefania Alcaraz², Raquel Horrillo², Javier Ramón-Azcón^{1,3}, and Francesco De Chiara¹¹ Biosensors for Bioengineering Group, Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain² Scientific Innovation Office, Grifols, Barcelona, Spain³ ICREA-Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain

The liver, a vital organ, faces acute and chronic insults that disrupt its normal function. Acute damage, caused by toxins or infections, triggers inflammation and necrosis. Chronic insults, such as alcohol abuse or viral hepatitis, lead to fibrosis, cirrhosis, and hepatocellular carcinoma, posing significant clinical challenges. Fibrosis is a hallmark of liver damage driven by the activation of hepatic stellate cells (HSCs). Understanding the mechanisms underlying acute and chronic liver damage is crucial for developing effective treatments. Traditional liver models face several limitations. 2D cultures cannot maintain liver phenotype and functions for extended periods, making it difficult to model chronic exposure. Additionally, replicating fibrosis in 2D cultures is challenging due to HSC activation on plastic or glass surfaces. As a result, 3D models have emerged as a more physiologically relevant cellular microenvironment for investigating disease progression, identifying potential therapeutic targets, and developing new drugs.

We developed a 3D liver using human hepatocytes (HepaRG), HSCs (LX-2), and monocytes (THP-1). The cells were encapsulated in a mixture of gelatin methacryloyl and carboxymethyl cellulose methacrylate, and lithium phenyl(2,4,6-trimethylbenzoyl)phosphonate as a photo-initiator. The 3D livers were kept in culture for up to 30 days in serum-free medium. They were challenged with acetaminophen and LPS (APAP-LPS), known hepatotoxic compounds, to recreate the pathophysiological phenotype of liver damage *in vitro*. Dexamethasone was used as an anti-inflammatory drug to test the ability of 3D livers to predict drug efficacy.

Extensive liver damage characterized by hepatic stellate cell (HSC) activation and proliferation was observed upon challenge with APAP-LPS. *In vivo*, these cells exhibited the myofibroblast phenotype typical of activated HSCs. Additionally, impaired gene expression of hepatocyte functionality markers was observed. The transition from monocytes to proinflammatory cytokine-releasing macrophages measured the inflammation level. Notably, dexamethasone demonstrated potent beneficial effects, reducing hepatocyte damage, inhibiting HSC activation, and decreasing collagen production. These results were observed in both acute (high APAP-LPS concentration/3 days) and chronic (low APAP-LPS concentration/30 days) models.

The 3D model presented here demonstrates its value as a versatile platform for drug screening in both acute and chronic liver damage scenarios. Its ability to reproduce critical features of liver pathophysiology, including hepatocyte functionality impairment, HSC activation, and inflammation, makes it a valuable tool for studying liver diseases and evaluating potential therapeutic interventions. Furthermore, the adaptability of this model for high-throughput screening provides an opportunity to accelerate the drug discovery process and improve patient outcomes in liver damage-related conditions.

FLASH presented by:

NAME: Mamatha Nijaguna

GROUP: Cellular and molecular mechanobiology

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Inhibiting mechanotransduction as a novel approach for oncology therapy

Mamatha Bangalore Nijaguna¹, Ignacio Viciano Gonzalo^{1*}, Borja Mateos³, Anabel-Lise Le Roux¹, Evelyn Coderch Bifet¹, Xavier Salvatella³, Pere Roca-Cusachs Soulere^{1,2}

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³ Institute for Research in Biomedicine (IRB), Barcelona, Spain,

*Former researcher at IBEC

Increased tissue stiffness is a prevalent feature in most solid tumors. Normally, tissues and cells sense the surrounding stiffness and transduce this mechanical signal into biological activity through the process of mechanotransduction. This process is tightly regulated to maintain tissue homeostasis. In the case of solid tumors, increased tissue stiffness enhances mechanotransduction, promoting tumor progression. Our lab has identified a crucial interaction between a target protein and its binding partner, which is vital for mechanotransduction. The interaction is triggered by force-induced target protein unfolding, which only occurs in stiff tissue, initiating mechanotransduction. We thus propose a novel approach targeting mechanotransduction by inhibiting the stiffness-induced unfolding of the target protein. This approach is unconventional and has therapeutic application in several cancer types and beyond cancer.

Towards this goal, we have developed a thermal shift assay for our target and conducted High-throughput Screening (HTS) of ~5,000 molecules. This approach resulted in 6 confirmed hit compounds that thermally stabilize the target protein; of which, for 4 compounds, the NMR data suggested specific binding to the target protein. Beside HTS, we also used structure-based virtual screening to identify hits, which resulted in one hit molecule with confirmed target protein binding by NMR. We aim to valorize these hits for oncology therapy application. As an alternate approach, we designed both poly glutamine modified, and staple peptides based on the bonafide binding partner to our target. Both these peptides showed improved alpha helicity compared to WT peptide and shows specific binding to target protein by NMR. However, only the staple peptide thermally stabilized the target protein with improved binding affinity compared to WT. Hence it is a promising hit to validate in further studies along with small molecule hits. At present, we have work ongoing to validate the target protein specific hits in relevant *in vitro* and cellular assays to delineate the mechanism of action. Finally, we plan to evaluate the efficacy of lead molecules *in vivo* mouse breast cancer model system.

The outcome of this study will be a first-in-class mechanoinhibitor with therapeutic applications in oncology and various other pathological conditions. This research not only holds promise for developing innovative cancer therapies but also provides tool compounds that contributes to advancing our understanding of fundamental mechanobiology processes.

FLASH presented by:

NAME: Pau Guíllamat

GROUP: Integrative cell and tissue dynamics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Guidance of cellular nematics into self-shaping active surfaces

Guíllamat, Pau¹, Mirza, Waleed², K. Bal, Pradeep³, Gómez-González, Manuel¹, Arroyo, Marino³, Trepát, Xavier¹¹ Institute for Bioengineering of Catalonia (IBEC), Barcelona, Catalunya.² European Molecular Biology Laboratory (EMBL), Barcelona, Catalunya.³ LaCaN, Universitat Politècnica de Catalunya (UPC), Barcelona, Catalunya

The ability to harness the contractility from cells and tissues has opened the door to engineering synthetic living materials with emergent mechanical properties^[1–3]. Despite recent efforts for leveraging cytoskeleton-driven forces in biohybrid devices^[3], achieving efficient control of the cells' mechanics remains still a remarkable challenge. Here, by drawing inspiration from tissue morphogenesis^[4], we program contractile cellular sheets to generate specific shape transformations driven by supracellular force patterns fueled by cytoskeletal contractility. In particular, we use cellular nematics – tissues composed of elongated cells – where the organization of subcellular forces is dominated by cell orientation in nematic-like ordered domains, and the presence of topological defects, areas where order is lost^[5]. By directly controlling cellular orientation and topological defects, which can provide essential mechanical cues for tissue remodeling^[6–9], we organize millimeter-scale cell monolayers with programmed multicellular tension patterns that can be released via out-of-plane deformations to form reproducible three-dimensional shapes. This strategy will not only enable the mapping of morphogenetic events within living tissues, but also potentially lead to applications in tissue engineering^[10] and soft robotics^[3].

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FLASH presented by:

NAME: Annalisa Calò

GROUP: Nanoscale bioelectrical characterization

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Mechanical phenotyping of lung cancer CAFs

*Annalisa Calò**Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST),
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The tumour microenvironment (TME) is gaining progressive attention for its physical-chemical properties, favouring cancer spreading and therapeutic resistance. Among TME components, cancer-associated fibroblasts (CAFs) are critical players, whose features are not fully characterized (1). In our work, we use AFM-based mechanical mapping to study CAFs from two distinct subtypes of non-small cell lung cancer (NSCLC). Using optimized measurement protocols (2), we achieved a robust CAFs mechanical characterization (Y, adhesion force) across millimetre-size cell layers. The obtained results show significant differences among the two patients' groups, even in morphologically similar CAFs cells. These results show the potential of the AFM technique for highly sensitive functional characterization of primary cells, thus making it an interesting tool for clinical applications.

FLASH presented by:

NAME: Clément Hallopeau

GROUP: Integrative cell and tissue dynamics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Mechanisms of mechanical compartmentalisation in intestinal organoids

Clément Hallopeau ¹, Gerardo Ceadá ¹, Anghara Menéndez ¹, Sergio Palomo ², Carlos Perez Gonzalez ³, Marija Matejčić ¹, Eduard Batlle ², Xavier Trepac ¹

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Monolayers of intestinal organoids recapitulate the functional compartmentalisation seen in-vivo. Crypt-like regions host stem cells, Paneth cells and transit amplifying cells, whereas villus-like regions contain differentiated cells. Measurements of traction forces in these organoids have established that stem cells push the underlying substrate while the transit-amplifying cells pull it, defining clear mechanical and functional compartments (Pérez-González, Ceadá et al, Nat Cell Bio, 2021). Crypt compartmentalisation is attributed to a gradient in Eph/ephrin signaling, but how this gradient is linked to the mechanical pattern is unknown. To address this question, we studied the mechanical and functional compartmentalisation in organoids derived from mice lacking EphB2 and EphB3 (EphB2^{-/-}, EphB3^{-/-}). We found that, unlike in wild type organoids (WT), crypts of EphB2^{-/-}EphB3^{-/-} organoids (KO) expand at the expense of the villus-like region. This phenotype is associated to an increased proliferation of the KO crypts. In mechanical terms, the 3D traction pattern of the KO crypts is qualitatively similar to the WT, but forces have a decreased amplitude, suggesting a decreased tension around the KO crypts. Taken together, these data establish a link between the mechanical features and the size homeostasis of the functional compartments, governed by Eph/ephrin signaling in intestinal organoids.

FLASH presented by:

NAME: Guillermo Martínez Ara

GROUP: Integrative cell and tissue dynamics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

An optogenetic toolset to understand and control epithelial mechanical balance

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Cells form tissue structures through the interaction of mechanical forces¹. Synthetic biology proposes the control of such forces to improve our understanding of how tissue structures arise². In addition, optogenetics has opened the possibility of gaining spatio-temporal control of mechanical forces with light³. These approaches have proven to be useful for the study of epithelial morphogenesis^{4,5}. However, the experimental control achieved doesn't account yet for all the forces proposed in physical models of tissue morphogenesis. Several theoretical studies propose an epithelial mechanical balance between apical, lateral, and basal contractility⁶. In this project, we make use of optogenetic and synthetic approaches to gain control over this set of forces (apical, basal, and lateral contractility) to test whether they are sufficient to understand and control the shape of different epithelial cell types.

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FLASH presented by:

NAME: Miguel González Martín

GROUP: Cellular and molecular mechanobiology

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Designing mechanosensible molecules for the mechanical control of cellular transcription.

*Miguel González-Martín^{1,2}, Juan-Martín Portilla³, Marc Molina Jordán^{1,2},
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Cells sense mechanical signals in the process of mechanotransduction, activating pathways that govern cell behavior. However, it remains a challenge to engineer mechanotransduction pathways in a controllable and predictable manner. Here we aim to engineer a synthetic mechanosensitive transcription factor (msTTA). To this end, we exploit the force induced changes in nuclear transport, linking nuclear mechanical perturbations to gene expression. To do so, we are mechanically tuning the passive and facilitated transport properties of the synthetic msTTA. Through this we aim to recapitulate the localization behavior of endogenous mechanosensitive proteins such as YAP or Twist, but with a synthetic factor that activates genes of choice in a controlled way. Optimizing our reporter cells, we have set up a novel screening platform with substrates of different rigidity, from which we expect to identify highly mechanosensitive TF candidates that function in a tunable manner, as well as to elucidate which features make a transcription factor mechanosensitive. Overall, we expect to unlock precise transcriptional control through mechanical forces, and a state-of-the-art directed evolution platform for msTTAs. With the simplicity of this engineered regulatory module, we expect to describe the minimal elements of mechano-regulation of gene expression, as well as enabling the use of mechanotransduction in gene circuits control. This will open the field to use mechanotransduction approaches for complex synthetic biology applications.

FLASH presented by:

NAME: Gergo Matajsz

GROUP: Molecular imaging for precision medicine

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

RF Surface Coil Design for High-Throughput Metabolic Imaging using Microfluidics

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One of the most pressing challenges of modern healthcare is to determine the stages of complex diseases with a high level of accuracy. Current diagnostic methods (e.g., physical exam and screening mammogram for breast cancer), lack patient specificity and robustness, both of which are detrimental in early diagnostics and in treating the underlying problems.^[1] Organ-on-a-chip (OOC) platforms provide a promising low-cost and high-throughput alternative, as observing diseases in a physiologically accurate environment creates ground for better assessment. By combining these microfluidic systems with Hyperpolarized Magnetic Resonance Spectroscopic Imaging (HP-MRSI), we administer labelled compounds (e.g., ¹³C-labelled pyruvic acid) to our biological models located in one of our 8 experimental chambers^[2]. We polarize our samples with Dynamic Nuclear Polarization (DNP) by cooling to 1.4K, irradiating with microwaves and dissolving them into a buffer solution. When added to our biological models, the labelled compounds go through metabolism, and we acquire decaying signals of low-concentration metabolic products which blend in with the noise under normal circumstances. Therefore, we have a tool to study *in vitro* models that closely mimic the behaviour of tissue conditions. Despite all the advantages outlined above, two crucial challenges remain: efficiently acquiring the quickly decaying hyperpolarized signal and acquiring signals from several samples in parallel.

We use radiofrequency (RF) coils to pick up signal in our 3T MRI scanner. With commercially available coils, maximizing the filling factor of the device is difficult, as they are specifically designed for rodents rather than microfluidic platforms. Birdcage volume coils produce shorter peaks on the chemical shift imaging (CSI) spectrum, indicating weaker signal reception. Moreover, most surface coils designed for rodents are either too small to cover our desired region of interest (ROI) or are only sufficient for ¹H-MRSI. Our solution to this problem is maximizing the signal-to-noise ratio (SNR) with a custom-built surface coil, tuned to ¹³C at 3T (32.1 MHz) and matching the size of the microfluidic chambers. The coil will have a separate array element covering each chamber, and due to the thin platform and the negligibly small barrier between the coil and the samples, penetration depth becomes less of a concern. A parallel array will ensure the best signal transmission- and reception in each chamber, without the extra time required for gradient encoding necessary for phased arrays. Upon the completion of this coil, we expect to see our most robust signal detection system up to date with the highest SNR, showcasing the full potential of HP-MRSI to detect labelled compounds in parallel.

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32 INSTITUTE FOR BIOENGINEERING OF CATALONIA (IBEC)

17th IBEC SYMPOSIUM · BIOENGINEERING FOR EMERGENT AND ADVANCED THERAPIES

FLASH presented by:

NAME: Yolanda Castillo Escario

GROUP: Biomedical signal processing and interpretation

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Measuring High-Resolution Sleep Position and its Variability in Adolescents with Smartphone Accelerometers

*Castillo-Escario, Yolanda^{1,2,3} and Jané, Raimon^{1,2,3}*¹ *Universitat Politècnica de Catalunya - BarcelonaTech (UPC)*² *Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST)*³ *Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN)*

Sleep position affects sleep quality and the risk and severity of different diseases. Classical methods to measure sleep position are complex, expensive, and difficult to use outside the laboratory. Wearables and smartphones can help to address these issues to track sleep position in free-living conditions. Here, we monitor high-resolution sleep position in a large sample of adolescents (N=145) over several nights (1-9) using smartphone accelerometers. This study is part of the Research, Creation and Service program, a Citizen Science project by IBEC and the Education Department of the Government of Catalonia intended for high-school students. We aim to 1) investigate the distribution of sleep positions and position changes in adolescents, 2) study their variability across nights, and 3) propose new measures related to nocturnal body movements.

We developed a new index, the mean sleep angle change per hour, and compared it with classical measures, such as the number of position shifts per hour, the mean time at each position, and the number of periods of immobility. Our results indicate that, overall, participants spent 49% of the time on the side (25% right and 24% left), 36% in supine, and 15% in prone position. This is a higher amount of time in supine position and less in prone than children (3-12 years old), but more time in prone position and less on the side than adults. Moreover, adolescents moved more than adults during sleep according to all measures. There was some variability between nights, but lower than the inter-subject variability. In conclusion, this work systematically analyzes sleep position over several nights in adolescents, a largely unstudied population, and offers a new tool for monitoring high-resolution sleep position and its relationship with sleep quality in a simple and cost-effective way.

FLASH presented by:

NAME: Shuqin Chen

GROUP: Smart nano-bio-devices

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Convective Dynamics of Swarming Enzymatic Nanomotors

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Enzymatic nanomotors harvest kinetic energy through the catalysis of chemical fuels. When a drop containing nanomotors is placed in a fuel-rich environment, they assemble into ordered groups and exhibit intriguing swarming behaviour akin to the self-organization observed in bacterial colonies, bioconvection of aerobic microorganismal suspensions, and the coordinated movements of fish, ants, and birds. This swarming behaviour presents numerous advantages compared to individual nanomotors, including expanded coverage and prolonged propulsion duration. However, the physical mechanisms underlying the swarming have yet to be fully elucidated. Our study investigates the formation of enzymatic swarms using experimental analysis and computational modeling. We show that the directional movement of enzymatic nanomotor swarms is due to their solutal buoyancy. We investigate various factors that impact the movement of nanomotor swarms, such as particle concentration, fuel concentration, fuel viscosity, and vertical confinement. We examine the effects of these factors on swarm self-organization to gain a deeper understanding. In addition, the urease catalysis reaction produces ammonia and carbon dioxide, accelerating the directional movement of active swarms in urea compared with passive ones in the same conditions. The numerical analysis agrees with the experimental findings. Our findings are crucial for the potential biomedical applications of enzymatic nanomotor swarms, ranging from enhanced diffusion in biofluids and targeted delivery to cancer therapy.

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FLASH presented by:

NAME: Núria Blanco-Cabra

GROUP: Bacterial infections: antimicrobial therapies

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Novel Fluidic System With Controlled Shear Stress For Personalized Diagnostic In Biofilm-Related Infections

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Biofilms are complex microbial communities that exhibit enhanced resistance to antibiotics, posing significant challenges in treating chronic infections and necessitating personalized diagnostics for each case. The *in vitro* biofilm device (IVD) is a novel fluidic system designed to mimic dynamic conditions and control shear stress, better simulating *in vivo* environments. This device offers a straightforward and reproducible method to cultivate biofilms and assess treatment efficacy, enabling personalized strategies for managing biofilm-associated infections.

FLASH presented by:

NAME: Luisa Camerin

GROUP: Nanoprobes and nanoswitches

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Photoswitchable Carbamazepine Analogs for Non-Invasive Neuroinhibition *In Vivo*

Luisa Camerin^{1,2,3}, Galyna Maleeva^{1,2,*}, Alexandre M. J. Gomila^{1,2,*}, Irene Suárez-Pereira^{4,5,6}, Carlo Matera^{1,2,7}, Davia Prischich^{1,2,*}, Ekin Opar^{1,2}, Fabio Riefolo^{1,2,*}, Esther Berrocoso^{4,5,6}, and Pau Gorostiza^{1,2,8,*}

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A problem of systemic pharmacotherapy is off-target activity, which causes adverse effects. Outstanding examples include neuroinhibitory medications like antiseizure drugs, which are used against epilepsy and neuropathic pain but cause systemic side effects. There is a need for drugs that inhibit nerve signals locally and on-demand without affecting other regions of the body. Photopharmacology aims to address this problem with light-activated drugs and localized illumination in the target organ. Here, we have developed photoswitchable derivatives of the widely prescribed antiseizure drug carbamazepine. For that purpose, we expanded our method of ortho azologization of tricyclic drugs to meta/para and to N-bridged diazocine. Our results validate the concept of ortho cryptoazologs (uniquely exemplified by Carbazopine-1) and bring to light Carbadiazocine (8), which can be photoswitched between 400-590 nm light (using halogen lamps and violet LEDs) and shows good drug-likeness and predicted safety. Both compounds display photoswitchable activity *in vitro* and in translucent zebrafish larvae. Carbadiazocine (8) also offers *in vivo* analgesic efficacy (mechanical and thermal stimulus) in a rat model of neuropathic pain and a simple and compelling treatment demonstration with non-invasive illumination.

FLASH presented by:

NAME: Marta Badia

GROUP: Protein phase transitions in health and disease

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

A comprehensive landscape of IAPP amyloid aggregation

*Marta Badia, Benedetta Bolognesi**Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain.*

Amyloids formed by the islet amyloid polypeptide hormone (IAPP) have been shown to cause pancreatic beta-cell damage, leading to a decline in insulin secretion and Type-II diabetes (T2D).

Even minor changes in the IAPP primary sequence can influence its aggregation rate. IAPP sequence in bears, which only differs in four amino acids from human IAPP, does not form amyloids. Conversely, specific single amino acid changes are enough to accelerate the IAPP aggregation rate.

Deciphering how mutations modify IAPP aggregation can help us gain a mechanistic understanding of the process of amyloid formation of this peptide and preventively identify mutations that could increase the risk of developing T2D.

Here, we measured the impact of 1668 IAPP mutations on its ability to nucleate amyloids thanks to a multiplexed cellular assay.

Our dataset includes distinct types of mutations (substitutions, insertions, and deletions) and identifies a continuous stretch of residues (15-32) which likely builds the core of IAPP amyloids. This stretch matches the core of the first protofilaments that appear in the *in vitro* aggregation reaction. Inside this core, we find that mutations have a more drastic effect in the 22-27 NFGAIL segment, which is located in the interface between the protofilaments in IAPP fibrils.

Additionally, this mutational atlas also identifies many mutations in the N-terminal region of the peptide that increase amyloid formation, suggesting that local structural elements in this region can normally protect against aggregation.

FLASH presented by:

NAME: Cátia D. F. Lopes

GROUP: Molecular bionics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Precision nanomedicine-enabled CRISPR-powered gene therapy for efficient amyloid- β clearance across the blood-brain barrier

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder that primarily affects the brain, leading to cognitive decline and memory impairment. Its exact cause is not fully understood, but AD is characterised by the abnormal extracellular accumulation of amyloid- β protein aggregates and intracellular tau tangles, which disrupt normal brain functioning. AD has no cure, and the available treatments have limited effectiveness in halting or reversing the progression of the disease. In addition, with the growing global ageing of the population, AD incidence and prevalence are expected to double in Europe by 2050. Therefore, AD remains a significant global health challenge, awaiting the development of a reliable, safe, and effective therapeutic option to halt disease progression and prevent cognitive failure.

Aiming to improve AD pathophysiology, our team is developing an innovative solution to modulate the process of amyloid- β clearance through the blood-brain barrier (BBB). We are developing a pioneering gene therapy that integrates CRISPR/Cas9 technology to modulate the expression of a key intervenient in amyloid- β clearance at the BBB, thus enabling the transport of amyloid- β across it. Besides leveraging the precision of CRISPR/Cas9, this innovative strategy also incorporates super-selective nanocarriers tailored to target BBB and mediate the targeted therapeutic gene delivery. By synergistically combining advanced genetic and nanotechnology strategies, our approach is anticipated to influence AD progression positively.

Here, we will present our latest *in vitro* findings. Our results show a successful modulation of target gene expression levels in brain endothelial cells – a critical step in our intervention – and a consequent significant improvement in amyloid- β transcytosis through the brain endothelium.

CRISPR Cas9 technology's precision, coupled with our nanocarriers' specificity, can potentially impact the progression of AD by influencing the process of amyloid- β clearance at the BBB. These findings contribute to our understanding of AD pathophysiology and mark a significant stride towards developing a reliable, safe, and effective therapeutic option.

FLASH presented by:

NAME: Antonino Nicolò Fallica

GROUP: Nanomalaria

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Development of YAT2150 analogues as potent multistage antiplasmodial agents

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Malaria ranks among the most lethal parasitic diseases worldwide, with treatment efficacy declining due to increasing drug resistance against current antimalarial therapies. Addressing this issue requires identifying new compounds that disrupt multiple pathways critical to the parasite's infectiousness and survival. YAT2150, a fluorescent dye traditionally used to detect protein aggregation *in vitro*, has shown potential in this regard. Our research team discovered that YAT2150 effectively impairs protein aggregation in *Plasmodium*, thereby exerting an antiplasmodial effect^[1]. Encouraged by these findings, we initiated a lead optimization campaign to develop more potent and less toxic derivatives of YAT2150. We identified three promising derivatives that have low toxicity for human umbilical vein endothelial cells and Caco-2 cells and exhibit enhanced antiplasmodial activity against both asexual and sexual stages of *Plasmodium*. Additionally, these compounds are strongly active against *Plasmodium falciparum* strains resistant to current antimalarial drugs. Their effect in reducing protein aggregation in live *P. falciparum* cultures was assessed using the thioflavin-T assay. The compounds demonstrated favorable *in vitro* pharmacokinetic and pharmacodynamic profiles, and preliminary assays in a *Caenorhabditis elegans* model indicated for all of them a low *in vivo* toxicity. Overall, these findings highlight the potential of YAT2150 derivatives as promising candidates for the development of new antimalarial therapies.

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Funding:

This work was supported by grants PID2021-128325OB-I00, PID2020-118127RB-I00, and PDC2022-133085-I00, funded by Ministerio de Ciencia e Innovación/Agencia Estatal de Investigación (MCIU/AEI/10.13039/501100011033), which included FEDER funds.

FLASH presented by:

NAME: Nina Kostina

GROUP: Bioinspired interactive materials and protocellular systems

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Harnessing nature's blueprints to design interactive synthetic cells

*Nina Kostina, César Rodríguez Emmenegger
IBEC Institute for Bioengineering of Catalonia*

Nature achieves unmatched functionality by the self-assembly of (macro)molecular building blocks in a hierarchical manner. All information necessary for the function is encoded at the molecular level. Inspired by Nature, my research focuses on building synthetic cells (SynCells), tailor-made synthetic vesicles capable of recapitulating some fundamental biological properties and performing tasks. In particular, I am interested in building SynCells that interact with natural cells to direct their fate in a programmed manner and fight pathogens. To tackle this, we have designed and synthesized new families of amphiphiles —comb-polymers and Janus dendrimers— that self-assemble into cell-mimetic vesicles termed combisomes and dendrimersomes. Although these molecules do not exist in Nature, the formed vesicles closely mimic cell membranes' thickness, flexibility, and lateral 2D organization. The unparalleled matching of biophysical properties enabled the harboring of functional components of natural membranes and even fusion with living cells to “hijack” their periphery providing an almost inexhaustible palette to design the chemical and biological makeup of the synthetic cells. The final goal is that the SynCells will recognize the bacteria, engulf it and kill it inside the endosome. Such Phagocytic SynCells can serve as active scavengers that protect implants and medical devices from bacterial colonization using mechanisms that do not cause the emergence of resistance. This concept has never been explored before, and I believe that it has a very high potential to become a new strategy for combating antibiotic-resistant bacteria and will have a high impact on medicine.

FLASH presented by:

NAME: Claudia Codano

GROUP: Molecular bionics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

A fumarate-based nanomedicine for macrophages' phenotypic modulation

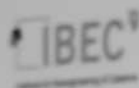
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The inflammatory process aims to eliminate cell injury causes, clear out damaged cells and tissues, and initiate tissue repair. However, dysregulated or chronic inflammation can lead to diseases, including autoimmune disorders, chronic infections, and certain cancers. M0 macrophages ("resting") play a central role in inflammation by adopting M1 ("pro-inflammatory") or M2 ("pro-resolving") phenotypes, determining the release of specific cytokines and mediators that shape the inflammatory response. Current strategies exploit metabolic reprogramming to modulate macrophage polarization and the release of inflammatory mediators via specific pathways. Nrf2 functions as an activator of antioxidant and detoxification processes and significantly influences mitochondrial and intermediary metabolism as part of its cytoprotective role. Fumarate derivatives, such as FDA-approved dimethyl fumarate (DMF), can modulate Nrf2, aiming to reprogram macrophages from an M1 to an M2 phenotype and restore the immune response. Our goal is to suppress inflammation dysregulation by leveraging the anti-inflammatory properties of fumarate combined with selective targeting of macrophages using a supramolecular nano-drug. We combined the fumarate derivative poly(propylene fumarate) (PPF) with poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) to create PMPC-PPF copolymer. PPF is a linear biodegradable polyester that releases fumarate in the cell milieu upon hydrolysis of its ester linkages. Previous studies from our group showed that monocyte-derived macrophages selectively internalize PMPC-decorated polymersomes after intravenous injection due to their interaction with receptors SRB1, CD36, and CD81, which are highly expressed on the cell surface.

PPF was synthesized by step-growth polymerization, and PMPC was added via reversible addition-fragmentation chain-transfer (RAFT). The copolymer was analyzed by nuclear magnetic resonance (NMR) to ensure quality and conversion grade. PMPC-PPF nanoparticles were obtained by solvent-switch and characterized by Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM) to evaluate their hydrodynamic diameter and morphology.

Preliminary studies on THP1-derived macrophages treated with PMPC-PPF nanoparticles showed a reduction in TNF- α expression, a key pro-inflammatory cytokine, along with an increase in IL-10, a cytokine primarily released by pro-resolving phenotypes. These findings were validated using primary macrophages, which also exhibited reduced pro-inflammatory cytokine expression and increased anti-inflammatory cytokine expression. The results were corroborated by measurements of released cytokines and nitric oxide concentrations. Furthermore, PMPC-PPF nanoparticles were used as a prophylactic approach in an *in vivo* model of multiple sclerosis, showing a delay in disease onset compared to the untreated group.

In summary, our preliminary findings demonstrate the encouraging potential of PMPC-PPF copolymer in reducing pro-inflammatory cytokines, suggesting a promising strategy for precise metabolic modulation of inflammation.



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Temporal analysis of Bioimpedance and Respiratory Volume Signals during Inspiratory Loading

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INTRODUCTION

Thoracic bioimpedance is a non-invasive technique capable to measure impedance changes in the thorax. These impedance changes are mainly due to respiration and previous studies reported a linear relationship between the impedance changes and the respiratory volume changes measured in the normal breathing [1]-[5].

Despite of these studies showed that this linear relationship can be affected by

ELECTROCARDIOGRAPHY

MATERIALS AND METHODS

10 HEALTHY SUBJECTS

4 females

6 males

24 - 35

ACQUISITION

Autflow

INCREMENTAL INSPIRATORY LOADING PROTOCOL

WIP MANEUVER

SIGNAL PROCESSING

Signals were filtered

Delay estimation

Δ, which represents



Figure 1. The four electrode electrode configurations. Top left: the four electrode configurations were positioned on the chest and abdomen. Top right: the four electrode configurations were positioned on the chest and abdomen. Bottom left: the four electrode configurations were positioned on the chest and abdomen. Bottom right: the four electrode configurations were positioned on the chest and abdomen.

RESULTS

- For most of these subjects, the delay appeared when inspiratory loads were imposed, specially when the load was high.
- Behavior of the delay differed by subjects and by electrode configuration.
- During the highest load, we observed the delay more frequently in configuration A, whereas in the other configurations the delay were more dependent on the subject.

max Δ, for S1: 1.77s

S2: 1.04, S3: 1.02 and S10 Δ, below 0.7 s

The presented results showed that the delay produced in six of the ten subjects were related to changes in breathing condition and were dependent on electrode configuration geometry and thoracic location.

We conclude that the delays showed in some subjects could include information associated with the changes in breathing pattern.

REFERENCES

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Imbalance Impact on the Prediction of Complications during Home Hospitalization: A Comparative Study

Calvo^{1,2}, Isaac Cano⁴, Carme
Mendez⁴, Vicent Ribas⁵, Felip Miralles⁵,
Roca⁴ Raimon Jané^{1,2,3}

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Introduction

Home hospitalization (HH) is presented as a healthcare alternative providing high standards of care when patients no longer need hospital facilities, but still require active and complex care [1].

Key characteristics of home hospitalization programs: reduced healthcare-associated costs and improved patient's quality of life.

However, complications due to lack of continuous observation at home may occur.

Identification of patients who may not benefit from HH is key.



Blood tests have been proven to provide relevant prognosis information in many diseases [2].

- Hypothesis: patients not being eligible for HH programs could be identified through the construction of predictive models based on data from routine blood tests at the moment of admission.

Since conventional machine-learning methods are extremely sensitive to class imbalance and unsuccessful HH cases (eventually needing hospital facilities) are rare, they show a strong bias towards the majority class.

This study analyzes and compares several sampling strategies and their impact on classification performance, in this particular scenario.

Materials and methods

Population

Data (24 variables) from 1951 patients admitted to the HH program at Hospital Clínic de Barcelona (Spain), between 2012 and 2015.

Successful group: 101 patients eventually needing regular hospitalization due to complications of different origin.

	Successful (n=101)	Unsuccessful (n=101)	p-value
Age, years old	70.6 ± 15.0	72.9 ± 14.7	0.072
Male sex, n (%)	135 (62.3%)	66 (65.3%)	0.563
Main diagnosis, n (%)			
Cardiology	196 (10.8%)	26 (25.7%)	<0.001
Respiratory	573 (31.0%)	24 (23.8%)	0.156
Oncoology	146 (7.9%)	9 (7.9%)	1.000
Surgery	366 (19.8%)	15 (14.9%)	0.276
Acute	569 (30.8%)	28 (27.7%)	0.584

Values are mean ± standard deviation or number of observations (%).

Statistical analysis

Redundancy evaluation through correlation analysis between pairs of variables (Spearman).

Primary analysis of statistical differences between successful and unsuccessful cases for each variable (Mann-Whitney U tests).

Sampling

Random sampling (75%) and testing (25%) subsets, using a stratified strategy. Model training was only applied to the first subset.

The remaining subset was then used for classification performance quantification.

The study compares the following sampling techniques:



b) Random over-sampling



Synthetic Minority Over-sampling Technique (SMOTE)
Random Over-Sampling Examples (ROSE)



Results

Correlation analysis

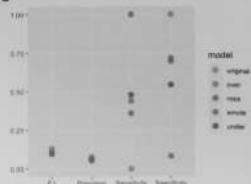
- Hematocrit was positively correlated with both hemoglobin concentration ($p = 0.98$) and red blood cell count ($p = 0.91$).
- Percentage and total amount of neutrophils ($p = -0.97$) and lymphocytes ($p = 0.96$) were highly correlated.

Statistical analysis

Statistically significant differences between successful and unsuccessful cases were observed for lymphocytes percentage ($p = 0.040$), hemoglobin concentration ($p = 0.030$), total amount of lymphocytes ($p = 0.023$), creatinine ($p = 0.023$) and red cell distribution width ($p = 0.002$).

Comparison of sampling approaches

- Original model heavily biased towards the majority class.
- All strategies showed low Precision:
 - Best Sensitivity: ROSE
 - Best F_1 : Random over-sampling



Conclusions

1. Significant correlations were noted among variables. Thus, a feature selection step would be advisable to minimize data redundancy.
2. Hemoglobin concentration, lymphocytes, red cell distribution width and creatinine were found to unmask statistically significant differences between patients undergoing successful and unsuccessful HH stays.
3. Among the analyzed sampling approaches, over-sampling strategies, such as ROSE and random over-sampling, showed the best performances. Nevertheless, further improvements should be proposed in the future.

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110	Core Facilities	MicrofabSpace and Microscopy Characterization Facilities: Empowering Research with New Technologies at IBEC

POSTER 1 presented by:

NAME: Gaia Amato

GROUP: Pluripotency for organ regeneration

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Developing Human Organoids To Model Genetic And Systemic Conditions During Congenital Anomalies Of The Kidney And Urinary Tract

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Congenital anomalies of the kidney and urinary tract (CAKUT) encompass a spectrum of malformations affecting the kidneys, urethra, ureters, and bladder during embryonic development. With an incidence of 4-60 per 10,000 births, CAKUT presents a significant challenge, primarily addressed through invasive treatments like urologic surgery, dialysis, or transplantation. Many of the 40 established monogenic causes of human CAKUT were initially derived as candidate genes from observations in mouse models of CAKUT and subsequently screened for their prevalence in human disease cohorts. However, the insights from mouse models, do not always directly translate to human genetics.

Human kidney organoids have emerged as crucial tools to study morphogenetic processes under healthy and disease states. When developed from human pluripotent stem cells (hPSCs), human kidney organoids represent an unprecedented tool set to study how mutations previously related to CAKUT can explain early disease phenotypes. Here we employed CRISPR/Cas9 to engineer hPSCs, creating reporter cell lines to monitor the endogenous expression of GATA3, a lineage specifier of one of the two stem progenitor cells of the kidney, namely the ureteric progenitor cells. At the same time, GATA3 represents one of the most prevalent genes leading to renal and extra-renal CAKUT manifestations in patients. Similarly, we have further generated knock-out (KO) lines in the background of the fluorescent reporter lines to investigate the impact of PAX2 and HNF1B mutations in the GATA3 lineage.

At the present time, we are assessing successful protein suppression in the KO backgrounds through Western blot and confocal microscopy analyses. Furthermore, we are validating the successful development of our CRISPR/Cas9 engineered lines through their differentiation into nephron-like kidney organoids. Importantly, we will investigate the impact of the different genetic backgrounds in extrarenal complications of CAKUT through the differentiation of these lines into cardiac and retinal organoids. The approach generated here will further allow for the investigation of maternal conditions leading to CAKUT such as gestational diabetes, hypertension, and hyperlipidemia.

POSTER 2 presented by:

NAME: Chiara Ninfali

GROUP: Biosensors for bioengineering

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Duchenne Muscular Dystrophy fibro-adipogenic progenitors impair muscle function of co-cultured healthy myotubes in a functional 3D model

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Duchenne Muscular Dystrophy (DMD) is the most prevalent pediatric neuromuscular disease, characterized by progressive skeletal muscle breakdown due to dystrophin protein deficiency. This results in muscle fiber loss, replaced by fibrotic tissue, hindering regeneration and treatment efficacy. Fibrotic tissue originates from fibro-adipogenic progenitors (FAPs), muscle stem cells capable of fibroblast differentiation and connective tissue production. Therefore, understanding the role of FAPs in DMD is crucial for therapy development. In this work we used an innovative 3D *in vitro* model using 3D-printed casting molds to encapsulate myogenic precursors from healthy individuals together with FAPs from healthy or DMD patients. These 3D tissues are fully functional skeletal muscles, capable of contraction and response to electrical stimuli. Through this co-culture model, we analyzed the fibrotic potential of DMD FAPs and we studied its impact on muscle functionality. Muscle tissues co-encapsulated with DMD FAPs exhibited increased fibrosis and reduced contractile capacity compared to those with control FAPs. Furthermore, they exhibited lower myofiber differentiation, likely due to fibrotic impediments in fiber fusion. This model promises insights into DMD fibrotic tissue representing a new tool for the development of new antifibrotic DMD treatments.

POSTER 3 presented by:

NAME: Karolina Zimkowska

GROUP: Molecular and cellular neurobiotechnology

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Monitoring Neuronal Activity in Human Cortical Organoids with Frontotemporal Lobar Degeneration-Tau (FTLD-Tau)Zimkowska, Karolina ^{1,2,3,4} Riu-Villanueva, Marc ^{1,2,3,4} Oliver-De La Cruz, Jorge ⁵ Roca-Cusachs, Pere ^{5,6} Lanciego, José Luis ^{3,7} Consiglio, Antonella ⁸ del Río, José Antonio ^{1,2,3,4}¹ Molecular and Cellular Neurobiotechnology Group, Institute for Bioengineering of Catalonia (IBEC), Parc Científic de Barcelona, 08028 Barcelona, Spain² Department of Cell Biology, Physiology and Immunology, Faculty of Biology, University of Barcelona, 08028 Barcelona, Spain³ Cibernet (Network Centre of Biomedical Research of Neurodegenerative Diseases), Institute of Health Carlos III, 08028 Barcelona, Spain⁴ Institute of Neuroscience, University of Barcelona, 08035 Barcelona, Spain⁵ Cellular and Molecular Mechanobiology Group, Institute for Bioengineering of Catalonia (IBEC), Parc Científic de Barcelona, 08028 Barcelona, Spain⁶ Faculty of Medicine, University of Barcelona, 08036 Barcelona, Spain⁷ CNS Gene Therapy Program, Center for Applied Medical Research (CIMA), University of Navarra, 31008 Pamplona, Spain⁸ Department of Pathology and Experimental Therapeutics, Bellvitge University Hospital-IDIBELL, Hospitalet de Llobregat, Barcelona, Spain; Institute of Biomedicine of the University of Barcelona (IBUB), Barcelona, Spain

Microtubule-associated protein tau is crucial in neuronal health by influencing axonal transport and microtubule stabilisation. In contrast, pathological tau is associated with early cognitive decline in Alzheimer's disease (AD) and pure tauopathies, such as FTLD-tau. Research shows that endogenous tau is implicated in neuronal activity (NA), although this role of tau is poorly understood. Neuronal excitation also regulates tau by promoting extracellular release and phosphorylation. Given that chronic epilepsy animal models show prolonged tau phosphorylation, emerging research is examining the role of pathological tau in epilepsy and the mechanisms underlying it and other tauopathy comorbidities. In this respect, changes in NA in the presence of tau mutations have not yet been analysed at presymptomatic stages. Furthermore, the currently available models lack the complexity of the human brain to fully understand the underlying mechanisms of tau pathology and its effects on NA. To address this, we present an *in vitro* platform utilising cortical organoids (COs) derived from hPSCs to unravel the impact of tau pathology on NA by exploring the changes that occur after the inclusion of mutated P301L-tau or full-length non-mutated human tau (2N4R) by adeno-associated virus. We employed calcium imaging techniques using genetically encoded calcium indicators and high-speed microscopy recordings to analyse changes in NA patterns and their correlation with tau pathology. Through overexpression of P301L-tau, we successfully developed COs that exhibit hyperphosphorylated tau as observed by biochemical analysis of phospho-tau(Ser422), PHF-1, and AT8, as well as tau aggregates observed by positive staining for thioflavin-S. Additionally, we have shown that these COs have NA with

observed changes in neuron firing frequency and network connectivity. Moreover, we have found that P301L-infected COs have reduced inter-spike intervals and that the neuronal network displays reverberating super bursts relative to controls. Our findings suggest that tau mutations alter the NA of COs by producing hyperexcitable networks susceptible to seizure-like activity. Thus, we believe that tau hyperphosphorylation could be responsible for the occurrence of seizures in the early stages of certain tauopathies like FTLD-tau and even AD. This model can, therefore, be used to further explore the functional consequences of tau mutation-mediated changes of NA in tauopathies.

POSTER 4 presented by:

NAME: Gulsun Bagci

GROUP: Biomaterials for regenerative therapies

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Cell-Derived Extracellular Matrices for 3D Breast Cancer ModelsGulsun Bagci¹, Barbara Blanco-Fernandez^{1,2,3}, Elisabeth Engel^{4,1,5}¹ Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Barcelona 08028, Spain.² I+D Farma Group (GI-1645), Department of Pharmacology, Pharmacy and Pharmaceutical Technology, Facultad de Farmacia and Instituto de Materiales (IMATUS), Universidade de Santiago de Compostela, Santiago de Compostela 15782, Spain.³ Health Research Institute of Santiago de Compostela (IDIS), Complejo Hospitalario Universitario de Santiago de Compostela, Travesa da Choupana sn., 15706 Santiago de Compostela, Spain.⁴ IMEM-BRT, Ciència i Enginyeria de Materials, Polytechnical University of Catalonia (UPC), 08019 Barcelona, Spain.⁵ CIBER en Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, 28029 Madrid, Spain.

Decellularized Extracellular matrices are new scaffolds for bioengineering of 3D tumor-ECM *in vitro* models. The tunable composition, properties, and structure of Cell-derived Matrices (CDMs) make them versatile and easy to use by using desired cell types. Moreover, deposition of ECM can be achieved by adding specific stimulants such as Macromolecular crowding (MMC) like Ficoll dextran sulfate or treating with hypoxia/starvation. Our aim is to fabricate 2D cultured CDMs from human dermal fibroblasts (hDFs) in the presence of MMC/Ascorbic acid/ TGFβ-1, which can be used as bioinks to generate tumor models for breast cancer research and for testing anti-cancer drugs such as doxorubicin.

In this study, we generated CDM from hDFs at 2D culture. To prepare CDMs, hDF cells were treated with Ascorbic acid (AA) or Ficoll, AA, TGFβ-1 combination for 2-weeks to increase the deposition of CDM. The ECM production before and after decellularization was evaluated by BCA, hydroxyproline, qRT-PCR, immunofluorescence and mass spectrometry. Values were normalized by the total DNA and decellularization was done by NH4OH-TritonX-100 solution. Methacrylated CDM was mixed with GelMA (5%) to recapitulate 3D models for MC7-cells, COL1 and only GelMA (5%) was used as control. They were cultured for 14 days and cell viability was determined by calcein-AM PI staining and cell morphology was determined by Phalloidin DAPI staining. Moreover, after 14 days incubation they were treated with different concentration of doxorubicin for 48h and their IC50 values were determined by Alamar Blue reagent. Moreover, young modulus of hydrogels were determined by compression test and bioprintability of GelMA (5%) was optimized.

Based on our results, for hDF cells; Ficoll, TGFβ-1 and Ascorbic acid combination, and also only ascorbic acid treatments increased total protein and total collagen production. Moreover, mass spectrometry results indicated that AA and Ficoll+AA+TGFβ-1 treatment significantly changed the composition of CDM in terms of collagen synthesis. MCF-7 cells formed

spheroids in GelMA(5%) and they were alive after culturing 14days . Mixing GelMA (5%) with CDM increased young moduli of hydrogels. IC50 of DOX for MCF-7 cells was higher in COL1 hydrogel than GelMA (5%).

In conclusion, we developed CDM from hDF cells at 2D by treating them different stimulants to tailor CDM composition for mimicking breast cancer TME.

Combination of CDM with GelMA(5%) enables bioprintability of CDM to obtain 3D tumor models with desired shape and architecture and a powerful platform for doxorubicin testing.

POSTER 5 presented by:

NAME: Ainhoa Ferret Miñana

GROUP: Biosensors for bioengineering

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

3D bioengineered liver for the study of acute and chronic hepatic damageAinhoa Ferret-Miñana¹, Estefanía Alcaraz², Raquel Horrillo², Javier Ramón-Azcón^{1,3}, and Francesco De Chiara¹¹ Biosensors for Bioengineering Group, Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain² Scientific Innovation Office, Grifols, Barcelona, Spain³ ICREA-Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain

The liver, a vital organ, faces acute and chronic insults that disrupt its normal function. Acute damage, caused by toxins or infections, triggers inflammation and necrosis. Chronic insults, such as alcohol abuse or viral hepatitis, lead to fibrosis, cirrhosis, and hepatocellular carcinoma, posing significant clinical challenges. Fibrosis is a hallmark of liver damage driven by the activation of hepatic stellate cells (HSCs). Understanding the mechanisms underlying acute and chronic liver damage is crucial for developing effective treatments. Traditional liver models face several limitations. 2D cultures cannot maintain liver phenotype and functions for extended periods, making it difficult to model chronic exposure. Additionally, replicating fibrosis in 2D cultures is challenging due to HSC activation on plastic or glass surfaces. As a result, 3D models have emerged as a more physiologically relevant cellular microenvironment for investigating disease progression, identifying potential therapeutic targets, and developing new drugs.

We developed a 3D liver using human hepatocytes (HepaRG), HSCs (LX-2), and monocytes (THP-1). The cells were encapsulated in a mixture of gelatin methacryloyl and carboxymethyl cellulose methacrylate, and lithium phenyl(2,4,6-trimethylbenzoyl) phosphonate as a photo-initiator. The 3D livers were kept in culture for up to 30 days in serum-free medium. They were challenged with acetaminophen and LPS (APAP-LPS), known hepatotoxic compounds, to recreate the pathophysiological phenotype of liver damage *in vitro*. Dexamethasone was used as an anti-inflammatory drug to test the ability of 3D livers to predict drug efficacy.

Extensive liver damage characterized by hepatic stellate cell (HSC) activation and proliferation was observed upon challenge with APAP-LPS. *In vivo*, these cells exhibited the myofibroblast phenotype typical of activated HSCs. Additionally, impaired gene expression of hepatocyte functionality markers was observed. The transition from monocytes to proinflammatory cytokine-releasing macrophages measured the inflammation level. Notably, dexamethasone demonstrated potent beneficial effects, reducing hepatocyte damage, inhibiting HSC activation, and decreasing collagen production. These results were observed in both acute (high APAP-LPS concentration/3 days) and chronic (low APAP-LPS concentration/30 days) models.

The 3D model presented here demonstrates its value as a versatile platform for drug screening in both acute and chronic liver damage scenarios. Its ability to reproduce critical features of liver pathophysiology, including hepatocyte functionality impairment, HSC activation, and inflammation, makes it a valuable tool for studying liver diseases and evaluating potential therapeutic interventions. Furthermore, the adaptability of this model for high-throughput screening provides an opportunity to accelerate the drug discovery process and improve patient outcomes in liver damage-related conditions.

POSTER 6 presented by:

NAME: Julia Fabà-Costa

GROUP: Bioengineering in reproductive health

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Development of an ex utero embryo culture platform to address post-implantation development and early placentation*Fabà-Costa, Julia¹, Godeau, Amélie L.¹, Casals, Marc¹, Massafret, Ot¹, Seriola, Anna¹, Ojosenegros, Samuel¹**¹ Bioengineering in Reproductive Health, Institute for Bioengineering of Catalonia (IBEC), 08028 Barcelona, Spain.*

Infertility is defined by the failure to conceive a child after 12 months or more of regular unprotected sexual intercourse, and affects between 12.6% and 17.5% of reproductive-aged couples worldwide. One of the critical points for pregnancy progression is embryo implantation, since the majority of unsuccessful conceptions are lost in this step and only one third of conceptions progress and lead to a live birth. This natural inefficiency of human implantation has not been significantly improved with the arrival of assisted reproductive techniques. Therefore, in order to overcome this roadblock in human reproduction, we need a deeper understanding of the sequences of events that control the formation and progression of the human conceptus during implantation. The current embryo culture technology patches different stages of embryo development, ranging from preimplantation stages to early organogenesis. But, so far, nobody has accomplished a continue culture from zygote all the way until organogenesis. The static culture conditions used in those systems, where the distribution of nutrients and gases depends only on passive diffusion, is one of the limiting factors. Here, we propose an embryonic culture platform integrated into a microfluidic device which allows us to apply a flow of media to the culture system. These features enable us to adapt both gases and nutrient supply to the developmental stage of the embryos, while also facilitating the removal of waste products. The device is fabricated by replica molding and consists of a culture platform with an integrated hydrogel layer on which the embryos can implant. Fabrication and sterilization protocols have been adapted to host mouse embryos. They have been cultured from blastocyst stage (day 4.5 after fertilization) to early-gastrulation stage after 4 days of culture in the device. Embryos have an implantation morphology similar to those shown in literature, and express Oct4, a marker of the embryonic epiblast, precursor to the future fetus, and Cdx2, a marker of the trophoblast, the extraembryonic tissue precursor of the placenta. Altogether, we have engineered a microfluidic platform that will help us to study mouse and human embryo implantation under controlled culture conditions enabling us to study embryo development from zygote to post-gastrulation stages.

POSTER 7 presented by:

NAME: Victoria Batto

GROUP: Associated researcher

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Identifying Clinically Relevant Biomarkers in NSCLC through Collagen Fragment Analysis*Victoria Batto¹, Neel I. Nissen², Nicholas Willumsen², Noemí Reguart³, Morten Karsdal², Jordi Alcaraz^{1,3}**¹ Unitat de Biofísica, Department of Biomedicine, School of Medicine, University of Barcelona, Barcelona, Spain**² Nordic Biosciences, Herlev, Denmark**³ Thoracic Oncology Unit, Hospital Clínic de Barcelona, Barcelona, Spain***INTRODUCTION & AIM**

Lung cancer is the leading cause of cancer-related death in both women and men, partly due to suboptimal therapies and lack of efficient biomarkers for patient stratification and early diagnosis. Most patients are diagnosed with non-small cell lung cancer (NSCLC), classified into adenocarcinoma (ADC), squamous cell carcinoma (SCC), and less frequent histologic subtypes. A hallmark of NSCLC is a fibrotic stroma rich in tumor-associated fibroblasts (TAFs) with excessive collagen deposition. Most TAFs exhibit an activated/myofibroblast-like phenotype, contributing to excessive collagen deposition. Collagen fragments may be produced during lung cancer progression due to pathologic extracellular matrix turnover. However, the specific collagen fragments produced by TAFs in NSCLC remain largely unknown.

METHODS

We analyzed collagen fragments secreted by TAFs from surgical ADC and SCC patients using ELISA with monoclonal antibodies reacting with the small parts of the protein fragment exposed after proteolytic cleavage. Fibroblasts from patient-matched uninvolved pulmonary tissue served as controls. Both TAFs and control fibroblasts (CFs) were stimulated with TGF- β 1 to regain the activated phenotype of TAFs found in patient samples, partially lost in culture.

RESULTS

Data from our patient cohort (n=20) revealed aberrant secretion of collagen fragments in TAFs from type I, III, and VI collagens, referred to as exPRO-C1, PRO-C3, and PRO-C6, respectively. Both lung cancer-associated fibroblasts (TAFs) and normal fibroblasts (CFs) exhibit higher exPRO-C1 levels compared to PRO-C3 and PRO-C6. Notably, TGF- β significantly elevates exPRO-C1 and PRO-C3 levels, with no effect on PRO-C6. TAFs consistently show higher levels of all measured collagen fragments compared to CFs. TGF- β induces higher exPRO-C1 levels in TAFs than in CFs. Additionally, SCC-TAFs exhibit higher exPRO-C1 levels than

ADC-TAFs, while PRO-C3 and PRO-C6 levels do not significantly differ between ADC and SCC. The shSMAD3 (SCC-like) and shSMAD2 (ADC-like) models exhibit trends consistent with their respective histological types.

CONCLUSIONS

An aberrant response to TGF- β in TAFs results in pathological production of exPRO-C1, PRO-C3, and PRO-C6. Elevated levels of exPRO-C1 and PRO-C3 could serve as reliable indicators of lung TAF activation *in vitro*. Higher collagen fragment levels in TAFs compared to CFs suggest these fragments as potential biomarkers for tumor presence. Importantly, exPRO-C1 emerges as a promising biomarker, potentially offering clinical relevance for distinguishing between NSCLC histological subtypes. Further investigation into the pathological functions of these collagen fragments in NSCLC and their potential clinical applications is essential.

POSTER 8 presented by:

NAME: Dakota Coloroso

GROUP: Biomaterials for neural regeneration

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Development of a 3D Organoid-on-a-Chip Device for Human Spinal Cord Models

*Dakota Coloroso¹, Palash Chandravanshi¹, Marta Cuenca², Marco Fusco¹, J. Alberto Ortega^{1,2}, Zaida Álvarez¹*¹ Institute for Bioengineering of Catalonia (IBEC), Barcelona 08028, Spain² Department of Pathology and Experimental Therapeutics, Institute of Neurosciences, University of Barcelona, Barcelona 08907, Spain

The current lack of *in vitro* human models that accurately mimic injury mechanisms relevant to spinal cord injury (SCI) significantly hinders our understanding and development of effective treatments. This study introduces an innovative 3D organoid-on-a-chip device designed to culture human spinal cord organoids (hSCOs) with a predefined geometry that closely resembles the native spinal cord. This advancement addresses the need for more effective models in SCI research. We designed and fabricated the device using cutting-edge 3D printing technology and tested the printability of three commercially available resins. Subsequent optimization of the 8-well device included performing computational flow analysis to ensure uniform media distribution and minimize cell disruption. We then evaluated the biocompatibility of the devices and investigated organoid behavior after injury. The response of injured hSCOs within the device provided insights into the effectiveness of simulating real-world SCI processes, including inflammatory response, neural cell death, and glial scar formation. We are now evaluating the reproducibility and scalability of our results to ensure the device's applicability for broader use in SCI research and therapeutic development. The success of this project will significantly advance SCI research by providing a more humane alternative to animal testing and enabling the creation of reproducible 3D SCI models.

POSTER 9 presented by:

NAME: Armando Cortés Reséndiz

GROUP: Biosensors for bioengineering

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Decoding skeletal muscle-liver axis in the context of sarcopenia: Towards the multi organ on a chip*Cortés-Reséndiz, Armando¹, De Chiara, Francesco¹, Fernández-Garibay, Xiomara¹, Fernández-Costa, Juanma¹, Ramón Azcón, Javier^{1,2}*¹ *Institute for Bioengineering of Catalonia, Barcelona, Spain*² *ICREA-Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain*

Sarcopenia is characterized by marked reductions in skeletal muscle mass and quality, which impacts the mobility and autonomy of patients. They suffer not only from low physical performance and strength but also face a greater risk of falls and further comorbidities, one of those being non-alcoholic steatohepatitis (NASH), as reported by some studies. These have described a correlation between diminished muscle strength and the onset of NASH. Thus, our study examines sarcopenic phenotypes in three-dimensional muscle tissues in contact with conditioned media from NASH.

This approach involves subjecting skeletal muscle tissues to incubation in culture media derived from a pre-established model of NASH. We encapsulated human hepatocytes and hepatic stellate cells (HSC) in a collagen-based hydrogel. After treatment, our model accumulates excess lipids upon a challenge with non-esterified fatty acids (NEFAs), shows activation of HSC, primary drivers of fibrosis, and exhibits a proinflammatory environment. Such conditions trigger an atrophic phenotype in healthy skeletal muscle tissues, fabricated by encapsulating human myoblasts in a Matrigel and fibrinogen matrix using PDMS casting. Skeletal muscle tissues were functionally evaluated as well by electrical pulse stimulation (EPS). We show that treated tissues exert lower contractile forces during EPS regime compared to our control conditions.

Both of our models pose valuable tools to aid in the identification of potential drug targets and therapeutic strategies, as they mimic key features and cellular microenvironments of sarcopenia and NASH. For this reason, our investigation marks a critical step toward understanding the intricate associations between these diseases. With the multi organ on a chip in sight, we will focus on integrating a sensing platform to our disease models. Recent advances from our research group make it possible to use nano plasmonic biosensors for the detection and measurement of proteins secreted to the culture medium.

POSTER 10 presented by:

NAME: David Bartolomé-Català

GROUP: Biomimetic systems for cell engineering

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Designing *in vitro* platforms to study transendothelial T cell migration in colorectal cancerDavid Bartolomé Català⁽¹⁾, Jordi Comelles⁽¹⁾, Aina Abad⁽¹⁾, María García-Díaz^(1, 2), Elena Martínez^(1, 2, 3)¹ Biomimetic Systems for Cell Engineering Laboratory, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain,² Department of Electronics and Biomedical Engineering, University of Barcelona (UB), Barcelona, Spain,³ Centro de Investigación Biomédica en Red (CIBER), Barcelona, Spain

Over the years, a better understanding of the tumor microenvironment (TME) in colorectal cancer (CRC) has highlighted its critical role in tumor development and progression. The infiltration of cytotoxic T lymphocytes into the tumor is one of the most predictive factors of prognosis in CRC. Therefore, great interest has been focused on understanding the ability of T lymphocytes to cross the endothelial barrier, navigate the stroma and access the tumor, and how the TME affects this migration. However, studying this process *in vivo* is extremely difficult and costly, and standard *in vitro* models are often too simplistic to fully recapitulate the journey of T cells from the blood stream to the tumor. To this end, we use a tiered approach developing increasingly complex *in vitro* models to dissect the mechanisms underlying transendothelial migration (TEM) and T cell motility within the stromal microenvironment. Specifically, we investigate distinct migration steps using: (i) a Transwell model cultured with endothelial cells mimicking the leukocyte endothelial transmigration, (ii) and primary T cells embedded in hydrogels mimicking the migration through the stromal compartment. To accurately reflect the signaling cascades present during the early stages of CRC, we use conditioned media from APC-/- intestinal organoids and compare T cell responses in these models with those in conditioned media from wild-type intestinal organoids. Finally, (iii) a Transwell model that combines the stromal compartment and intestinal organoid to effectively recapitulate the stromal T cell migration dynamics in the presence of an epithelium. Each of these models will help understand the effect of the different barriers and TME in the T cell migration, providing a toolbox to study the mechanical and biochemical signals during immune cell recruitment within a complex tissue-like CRC model.

POSTER 11 presented by:

NAME: Xiomara Fernández Garibay

GROUP: Biosensors for bioengineering

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Advancing Preclinical Research of Myotonic Dystrophy Type 1 with 3D Functional Human Skeletal Muscle Tissues

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Myotonic Dystrophy Type 1 (DM1) is a life-threatening disease and the most prevalent hereditary myopathy in adults. This disorder is mostly characterized by progressive muscle atrophy, weakness and hyper contraction. Conventional 2D cultures and animal models have enabled significant progress in understanding the molecular basis of DM1, leading to the discovery of potential therapies. However, these models cannot emulate the complexity of this disease and effective treatments remain elusive. Thus, our aim was to develop contractile DM1 human skeletal muscles that reproduce the molecular, structural, and functional phenotypes of the disease.

Our tissue engineering approach consists of encapsulating human immortalized myoblasts from three DM1 patients within biocompatible hydrogel scaffolds, suspended around two flexible posts. This system promotes cell differentiation into aligned 3D myotubes that can contract in response to electrical stimulation. We characterized the functionality of these bioengineered skeletal muscle tissues, assessing key functional parameters. Importantly, besides reproducing molecular and structural hallmarks of DM1, our 3D model presents the unique opportunity to reproduce fixed and transient weakness *in vitro*, DM1 phenotypes that could not be emulated without resorting to animal experiments. This achievement enables us to evaluate the contractile response of DM1 tissues upon treatment with candidate therapies for this disease. In summary, our 3D functional skeletal muscle tissues from DM1 patient-derived cells recreate critical disease phenotypes which provide more relevant insights into effective treatments, potentially accelerating therapeutic breakthroughs.

POSTER 12 presented by:

NAME: Judith Fuentes Llanos

GROUP: Smart nano-bio-devices

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Real-Time Force Monitoring of Electrically Stimulated 3D Bioengineered Muscle Bioactuators Using Organic Sensors with Tunable Sensitivity

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The contractile nature of skeletal muscle tissue makes it especially attractive for powering biohybrid actuators. Additionally, the evaluation of the contraction force provides information about the level of cell maturation and functionality, being one of the key features in drug screening platforms. Current efforts are devoted to integrating force measurements systems to obtain real-time information over the skeletal muscle tissue performance, going one step forward towards the automation of these biohybrid platforms.

Here, we integrated 3D-bioengineered muscle tissues with Organic Field-Effect Transistors (OFET)-based sensors to define a soft bioactuator with real-time force monitoring capabilities. The muscle tissue is electrically stimulated while the organic sensor ensures a transduction of the exerted force into an electrical signal that can directly be available for monitoring the performance of the bioactuator. Sensor calibration is carried out to define its sensitivity at different biasing conditions. With respect to conventional two-terminal piezoresistive strain gauges, OFET-based strain sensors employ a third terminal, the gate, which allows modulating the conductivity of the organic semiconductor and thus the sensing performances. Therefore, we are here proposing for the first time a programmable and complete electronic stimulation-feedback approach for monitoring biohybrid cell actuators. A complete evaluation of the sensing performance is presented, demonstrating that the real-time monitoring is effective at different conditions, including the frequency of the stimulation signal and the chemical modulation of the tissue contraction with caffeine and dantrolene, demonstrating its potential use as a drug testing platform.

Therefore, the use of (OFET)-based sensors is not only of interest for providing key information about the biological actuator, going one step forward towards its automation, but also to be used in other bioengineered muscle platforms for its versatility and easy integration.

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POSTER 13 presented by:

NAME: Steffen Grosser

GROUP: Integrative cell and tissue dynamics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

**Optogenetic control of collective dynamics
in epithelial cells***Steffen Grosser¹, Leone Rossetti², Ricard Alert³, Xavier Trepac^{1, 4, 5, 6}*¹ *Integrative cell and tissue dynamics, IBEC, Barcelona*² *Kings College, London, England*³ *Max-Planck-Institute for the physics of complex systems, Dresden, Germany*⁴ *Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Barcelona*⁵ *Facultat de Medicina, Universitat de Barcelona, Barcelona*⁶ *Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona*

Collective cell motility states such as flocking and jamming are active matter states that play important roles in development and disease. However, much of the physical dynamics of these collective states remains to be discovered.

We use optogenetics to control and understand collective cell dynamics. Optogenetics allows to control cell mechanics on-demand with unprecedented temporal and spatial precision by photoactivating the molecular machinery that regulates cell mechanics. Robust control of cell migration has not been achieved yet, and further, there is considerable uncertainty in how to create the force patterns that can move cells. We thus use traction force microscopy to measure cell forces and relate them to optogenetic activation and motility.

We show that optogenetic activation of the small GTPases Rac1 or CDC42 on one side of an epithelial cell induces lamellipodium growth in nearly all cases. It also lead to cell motility in the direction of the activated side, although with lower efficiency.

We find that cell motility additionally requires a traction force asymmetry, characterized by a strong traction peak on the leading edge and a broader distribution of traction at the trailing edge. While these forces always cancel out in total, an active gel model allows to decompose them into active tractions and dissipative forces. This supports the existence of a new, first force-velocity relation for cell motility. Further work will show how this can be employed to engineer collective cell motility.

POSTER 14 presented by:

NAME: Inés Martínez Soria

GROUP: Molecular and cellular neurobiotechnology

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Roles of the Adhesion G Protein-Coupled Receptor D1 (ADGRD1) during CNS development and adult neuronal plasticity

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ADGRD1 (GPR133) is an orphan receptor of the adhesion G protein-coupled receptor (GPCR) family. GPCRs are a large and diverse group of membrane receptors that play critical roles in cellular communication and signal transduction. The adhesion GPCR subfamily is characterized by their long N-terminal regions that contain various adhesion motifs, which are important for cell-cell and cell-matrix interactions. In addition, these receptors signal through the intracellular G- α family. This protein has been studied as a glioblastoma multiple (GBM) marker, due to its correlation between levels of ADGRD1 expression and GBM severity. However, physiological functions of this protein in healthy CNS are unknown. Thus, our aim is to study ADGRD1 involvement during neurodevelopment and neuronal plasticity. We have analyzed an Adgrd1-KO mice using immunohistochemical, RT-qPCR and behavioral techniques (nest building test, tail suspension test, open field test and novel object recognition test). In addition, we studied their kainate susceptibility with subsequent histological analysis (Fluoro-Jade B, c-fos, pERK, GFAP, DCX or Parvalbumin). In the analysis of the Adgrd1-KO mice, we observed some differences between our model and wild type mice, such as a higher expression of DCX in the hippocampal dentate gyrus. This result suggests a participation of ADGRD1 in the neural stem cells proliferation or differentiation. In addition, the kainate susceptibility experiments revealed a clear enhanced susceptibility to epilepsy in Adgrd1-KO mice. Indeed, treated mice showed increased cell death in the hippocampal region, astrocytes reactivity, and increased post-epileptic hippocampal neurogenesis. Therefore, these results highlight a role of ADGRD1 (GPR133) in neurodevelopment, neural plasticity and neuroprotection.

POSTER 15 presented by:

NAME: Dayaneth Jácome

GROUP: Molecular and cellular neurobiotechnology

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Targeting PrPC signaling involved in glioblastoma by miR-519a-3p as therapeutic interventionDayaneth Jácome^{1, 2, 4}; Tiziana Cotrufo^{1, 2, 3}; José Antonio del Río^{1, 2, 3, 4}; Rosalina Gavín^{1, 2, 3, 4}.¹ Molecular and Cellular Neurobiotechnology, Institute for Bioengineering of Catalonia, Barcelona, Spain.² Department of Cell Biology, Physiology and Immunology, University of Barcelona, Barcelona, Spain.³ Institute of Neuroscience, University of Barcelona, Barcelona, Spain.⁴ Center for Networked Biomedical Research in Neurodegenerative Diseases (CIBERNED), Barcelona-Madrid, Spain.

Glioblastoma multiforme (GBM) is one of the most lethal cancers in humans and the prognosis of affected people remains dismal. Current treatments are largely ineffective due to several significant challenges, such as late detection of the tumour, its rapid spread in the brain parenchyma that hinders surgical techniques, or the existence of the brain blood barrier, which limits the bioavailability of drugs and facilitates immune evasion, among others.

It is known that the Cellular Prion Protein (PrPC) overexpression in GBM, which also increase under hypoxic conditions, even worsens prognosis and promotes resistance to therapies. In fact, PrPC overexpression in different tumour types has been widely reported, often increasing tumorigenic potential, proliferation and invasion through STI-1, Bcl-2, GSK3-Wnt signalling pathways, among others.

Blocking the formation of the PrPC/STI-1 complex has already been shown to be an effective *in vitro* therapy for GBM development and overall, intervention on PrPC levels holds promise for future cancer therapies. In this sense, downregulation of PrPC by miR-519a-3p has already been demonstrated. Also, reduced expression levels of miR-519a-3p have been reported in GBM and the strategy of increasing miR-519a-3p levels using mimics has already been investigated, demonstrating significant improvements in reducing tumour proliferation, enhancing chemosensitivity and decreasing metastatic capabilities by inhibiting the STAT/Bcl-2 pathway.

Currently, although no miRNA-based therapy has yet been approved by the FDA, there are several phase I and II clinical trials examining the use of miRNAs as pharmacological agents. Thus, demonstrating *in vitro* reversion of PrPC signalling on STI-1, Bcl-2, GSK3-Wnt pathways through miR-519a-3p will further elucidate the antitumorigenic potential of this miRNA.

POSTER 16 presented by:

NAME: Sheeza Mughal

GROUP: Biosensors for bioengineering

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Transient Metabolic Adaptation and weakness in healthy 3-D *in vitro* skeletal muscle tissues exposed to Chronic Fatigue Syndrome and Long COVID-19 seraSheeza Mughal ^{1,2}, Félix Andújar-Sánchez ^{2,3}, María Sabater-Arcis ¹, Glòria Garrabou ³, Juan M. Fernández-Costa ¹, and Javier Ramón-Azcón ^{1,4}¹ Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST); Barcelona, Spain² Universitat de Barcelona³ Institut d'Investigacions Biomèdiques August Pi i Sunyer; Barcelona, Spain⁴ ICREA-Institució Catalana de Recerca i Estudis Avançats; Barcelona, Spain

Myalgic Encephalomyelitis, also referred to as Chronic Fatigue Syndrome (ME/CFS) and Post-Acute Sequelae of SARS-COV-2 infection (PASC, or Long COVID-19) are clinically challenging, multisymptomatic conditions with no consensus on disease progression or a diagnostic marker (1). Despite extensive research, there is no *in vitro* model in place to study progressive skeletal muscle wasting, peripheral fatigue or potential therapeutic targets. We developed 3-D *in vitro* skeletal muscle tissues to map the progression of functional, physiological, and metabolic adaptations of muscles in response to patient sera over time. During short exposure, we treated the tissues for 48 hours with patient and control sera. The contractile strength of muscles treated with patient sera was severely compromised. Transcriptomic analyses of short exposure samples showed an absence of significant differentially expressed genes between ME/CFS and LC-19. The analyses revealed an upregulation of glycolytic enzymes such as ENO3 and PDK4 at the gene level, as well as a decline in DNMI, involved in mitochondrial fission. Subsequent structural analyses confirmed hypertrophy in myotubes and hyperfused mitochondrial networks with an elevated Mitochondrial oxygen consumption capacity in ME/CFS conditions. Interestingly, at short exposures of 48 hours, the muscle tissues appeared to be adapting to the stress factors by upregulating glycolysis and increasing the muscular metabolic volume. Prolonging the exposure to 96 and 144 hours induced high fatiguability, and fragility in tissues. The mitochondria, at longer exposures, appeared to be fragmented and assumed a toroidal conformation indicating a change in mitochondrial membrane potential. We hypothesize that the disease progresses through an intermediary stress-induced hypermetabolic state, ultimately leading to severe muscular deterioration. This is the first account of research that proposes transient metabolic adaptation and compensation against stress in 3D skeletal muscles exposed to ME/CFS and Long COVID-19 patients' sera.

POSTER 17 presented by:

NAME: Adrià Noguera Monteagudo

GROUP: Biomaterials for regenerative therapies

INSTITUTION: Universitat de Barcelona

Advanced Microfluidic Platform For 3d Angiogenesis Studies

A. Noguera-Monteagudo¹, A. Vilche¹, B. Van Durme³, Y. Dong³, R. Rodríguez^{2,3}, S. Van Vlierberghe³, E. Engel³, O. Castaño^{1,2}

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Angiogenesis, the formation of new blood vessels from existing ones, is crucial in revascularizing implants, tissue engineering, cancer growth, and various diseases^[1]. Recent advancements in microfluidics have created highly controlled microenvironments, enabling *in vitro* models of angiogenesis to study its mechanisms^[2-3]. Additionally, two-photon polymerization allows for printing three-dimensional hydrogels with precise structures within these devices on a micrometer scale^[4]. This enables the study of 3D *in vitro* models with controlled environments, facilitating the investigation of different geometries on angiogenesis.

This study employs an angiogenesis-based platform designed in AutoCAD, consisting of a central chamber (1300 μm wide, 8800 μm long, 150 μm high) connected to two lateral channels (750 μm wide, 150 μm high), each ending with deposits (radius 2 mm). Microfluidic devices are made from PDMS using a master mold produced with SU8-350 photoresist (MicroChem) via standard photolithography techniques. Scaffolds composed of gelatin-based ink (HYDROTECH INX U100, BioInx, Ghent, Belgium) are processed via photo 3D printing.

By integrating microfluidics with photo 3D printing, gelatin-based ink scaffolds can be printed within the microfluidic device. These scaffolds feature channels of 75 and 100 μm in desired heights and directions, enabling precise control over the microenvironment and three-dimensional structuring. This advancement enhances the precision and fidelity of angiogenesis studies.

In conclusion, microfluidic technology promises to transform our understanding of angiogenesis by providing a meticulously controlled platform for mechanistic studies. Additionally, fabricating engineered three-dimensional structures within microfluidic devices via two-photon polymerization opens numerous opportunities.

Acknowledgments:

The authors thank the Spanish Ministry of Science and Innovation (MICINN), and the State Research Agency (AEI) with Projects PID2021-124575OB-I00 and PDC2022-133755-I00. Finally, we also appreciate the support of Research Foundation Flanders (FWO) (1SH3W24N)

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POSTER 18 presented by:

NAME: Marc Riu Villanueva

GROUP: Molecular and cellular neurobiotechnology

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Viral expression of tau with the P301L mutation induces tauopathy hallmarks on pluripotent stem cell-derived neuronal cultures

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Tauopathies are a group of neurodegenerative diseases, including Alzheimer's disease, characterized by the deposition in specific brain regions of the abnormally misfolded and aggregated microtubule-associated protein tau. One of the most studied pure tauopathy is the frontotemporal lobar degeneration-tau (FTLD-tau) that is caused by a point mutation in the exon 10 of the MAPT gene, P301L. This condition falls within the broader category of frontotemporal dementias (FTD) and can lead to significant cognitive decline and behavioral changes. FTDP-Tau is relatively rare compared to other forms of dementia, such as Alzheimer's disease. Generally, frontotemporal dementias, including FTDP-Tau, are estimated to account for about 5% to 10% of all dementias. Overall, since frontotemporal dementias and FTDP-Tau are less common than Alzheimer's disease, research efforts tend to focus on better understanding these conditions to develop more effective treatments and improve patient quality of life. Indeed, P301L or the P301S mutations has been used in the last decades to model FTDP-Tau pathology in mice models. However, the translation from mouse models to human models is still very distant. Human induced Pluripotent Stem Cells (hiPSCs) have the potential to generate human and physiologically relevant neuronal cultures with which to model disease and test therapeutic approaches. We have developed a human *in vitro* neuronal model for tauopathies, based on human

Embryonic Stem Cells (hESCs)- and hiPSCs- derived neuronal cultures in which we show induction of relevant tau pathology through viral transduction of tau with the P301L mutation. We used NGN2 overexpression, a reliable and efficient method, to obtain mature neurons within a short timeframe. Having obtained a mature culture, we infected the cells with an adeno-associated virus (AAV) expressing the P301L mutated form of tau and with a fluorescent reporter (tdTomato) that allows us to better analyze the viral induction of the pathology. Three weeks post-infection, our model shows clear signs of aggregation of abnormally phosphorylated tau, as shown by Western Blot and immunofluorescence analysis of tau phosphorylation epitope AT8 (pTau Ser 202/Thr205) or by the presence of MC1 antibody – which recognizes neurofibrillary tangles. We look to use this model for further experiments, such as using microfluidic devices to analyze tau spreading and analyzing the effect of tau mutations on neuronal activity.

POSTER 19 presented by:

NAME: Gisele Priscila Soares de Aguiar

GROUP: Biomaterials for neural regeneration

INSTITUTION: University of Barcelona

Harnessing Spinal Cord ECM Cues to Enhance iPSC-Derived Neuronal Maturation and Regeneration

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The extracellular matrix (ECM) is a critical but often overlooked component of the central nervous system (CNS) microenvironment that significantly influences neuronal maturation, signaling, plasticity and aging. Neuronal maturation remains a significant challenge for current human *in vitro* systems, limiting their utility for the study of CNS injury and disease, as well as for potential regenerative therapies. In order to reproduce adult microenvironments that could promote neuronal maturation *in vitro*, several methods have been developed, including the use of generic ECM proteins, such as laminin and fibronectin, 3D matrices, such as Matrigel, and astroglial cells as feeder layers. However, more knowledge is needed to understand spatiotemporal-specific signals in the CNS that are involved in neuronal maturation as well as in regeneration. This study aimed to establish a platform to identify developmentally relevant cues from the spinal cord ECM to enhance the maturation of iPSC-derived motor neurons. Our preliminary data indicate significant differences in the functional and regenerative capacities of the ECM derived from neonatal and adult mice. We also elucidated the differences in the spinal cord biochemical composition, a.k.a. matrisome, at these developmental stages and identified three potential candidates associated to perineuronal nets, which effectively promoted maturation of human iPSC-derived motor neuron cultures *in vitro*. Our results suggest that perineuronal nets not only modulate synaptic plasticity but promote neuronal maturation before perineuronal net assembly. Follow up studies will focus on developing precisely tailored materials to improve maturation and regeneration of injured CNS.

POSTER 20 presented by:

NAME: Aleixandre Rodrigo Navarro

GROUP: Microenvironments for Medicine

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Optogenetic gene expression control in *Lactococcus lactis*Rodrigo Navarro, Aleixandre⁽¹⁾, Salmeron Sanchez, Manuel^(1,2)¹ Institute for Bioengineering of Catalonia² Centre for the Cellular Microenvironment, University of Glasgow

Lactococcus lactis is a gram-positive bacterium that has been widely studied for its biotechnological and industrial applications due to its suitability to produce high-value chemicals and recombinant proteins. To this end, multiple chemically inducible gene and protein expression systems have been developed. Here we present a novel inducible system that relies solely on physical stimulus, specifically blue light, using a combination of small engineered Vivid photoreceptors from *Neurospora crassa* (enhanced Magnets or eMags) and a split T7 RNA polymerase.

Our findings demonstrate that the split T7 RNA polymerase is active and non-cytotoxic in *Lactococcus lactis*, and when fused to the eMags, it can drive gene expression in a light intensity- and time-dependent manner. This marks the first time that a split T7 RNA polymerase fused to photoreceptors has been described in a gram-positive bacterium.

This system provides an attractive alternative to chemically inducible systems since it can be activated by physical stimulus alone, thus avoiding the need to add inducers externally and improving its reversibility, without the need to remove the inducer from the medium. Furthermore, this system can be applied in various biotechnological applications where precise spatiotemporal control of gene expression is required, especially in the context of living biomaterials where chemical stimuli are diffusion-controlled, difficult to switch off and thus cannot be applied locally in a reversible way, a role where light excels. We foresee potential applications in tissue engineering and industrial protein production.

POSTER 21 presented by:

NAME: Gustavo San Miguel

GROUP: Biomaterials for regenerative therapies

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Production of Polyhydroxybutyrate (PHB) by *Bacillus cereus* 12GS for applications in regenerative therapiesGustavo de Jesus San Miguel Gonzalez ^{1,2}, Elisabeth Engel López ², Oscar Castaño Linares ², María Elizabeth Alemán Huerta ¹, Isela Quintero Zapata ¹, Raul Enrique Martínez Herrera ^{3,4}.¹ Instituto de Biotecnología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Av. Pedro de Alba y Manuel L. Barragán S N, San Nicolás de los Garza C.P. 66455, Nuevo León, Mexico² Biomaterials for Regenerative Therapies, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain³ Escuela de Ingeniería y Ciencias, Tecnológico de Monterrey, Av. Eugenio Garza Sada 2501 Sur, Tecnológico, Monterrey C.P. 64849, Nuevo León, Mexico⁴ Institute of Advanced Materials for Sustainable Manufacturing, Tecnológico de Monterrey, Av. Eugenio Garza Sada 2501 Sur, Tecnológico, Monterrey C.P. 64849, Nuevo León, Mexico

The necessity for bone repair in various patients who experience large bone resections or significant traumas has led to the development of bone grafts using tissue engineering approaches. To be widely utilized in clinical practice, bone tissue engineering products must overcome a series of challenges, with the complete supply of nutrients and metabolite diffusion being among the most critical. In the field of biomaterials, the electrospinning processing technique enables the production of polymeric fibers with diameters ranging from nanometers to micrometers, which are physically comparable to collagen fibers found in the natural extracellular matrix. Polymeric biomaterials, such as Polyhydroxybutyrate (PHB), have been previously employed in research for tissue engineering applications due to their useful mechanical properties, biodegradability, and biocompatibility. The production of PHB is carried out by various bacteria, such as *Bacillus cereus*, under unbalanced growth conditions, the bacteria are utilized as an energy reserve. Therefore, it is proposed to work with this biopolymer for its potential in such applications. Liquid GRPD (Glucose-Rich and Peptone-Deficient) culture media were prepared, adjusted to pH 8, sterilized at 121°C for 15 minutes, and inoculated with 100 µL of a 1×10^8 CFU/mL concentration of *Bacillus cereus* 12GS spores in a 250 mL flask containing 100 mL of the culture medium. The culture was incubated on a shaker at 30°C and 150 rpm for 24 hours. Subsequently, 4% of the culture was inoculated into new 500 mL Erlenmeyer flasks containing 200 mL of culture medium and placed on a shaker under the same conditions for 48 hours. Following this, the microbial biopolymer was extracted using a previously published methodology. A yield of 1.6 g/L of PHB, characterized by a white color and brittle appearance, was obtained. In total, 6.4 g of PHB was collected for future experiments. The polymer extract was analyzed using DSC and FTIR. Finally, it was dissolved and filtered with organic solvents to be used in an electrospinning technique to form scaffolds with potential applications in tissue engineering. The production of microbial biopolymers is competitive with petroleum-derived plastics due to their physicochemical properties, and when mixed with other polymers, they often enhance their thermal properties. Additionally, under optimal conditions, they can be applied in various areas of biomedical research.

POSTER 22 presented by:

NAME: Anisha Pahuja

GROUP: Pluripotency for organ regeneration

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Exploiting human pluripotent stem cells to study human disease in kidney and retinaAnisha Pahuja^{1,2}, Daniel Moya Rull¹, Elena Garreta^{1,2}, Nuria Montserrat^{1,2,3,4}¹ Pluripotency for organ regeneration. Institute for Bioengineering of Catalonia (IBEC), the Barcelona Institute of Science and Technology (BIST), 08028 Barcelona, Spain.² University of Barcelona, 08028 Barcelona, Spain.³ Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina, Barcelona, Spain.⁴ Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

Congenital anomalies of the kidney and urinary tract (CAKUT) comprise a wide range of structural and functional malformations affecting the kidneys and lower urinary tract, caused by genetic and environmental factors disrupting embryonic development. While mouse models have identified key players in CAKUT phenotypes, species-specific differences challenge the translation of these insights to human genetics. Human pluripotent stem cell (hPSC)-derived organoids provide a promising alternative for studying early morphogenetic changes related to CAKUT.

Our project aims to use hPSC-derived organoids to understand human kidney morphogenesis and CAKUT. Specifically, using our established kidney organoid-generation protocol, we will investigate impact of deletion of an imperative gene LHX1, which is involved in the induction of intermediate mesoderm and necessary for renal progenitor cell specification. The LHX1 knock-out organoids, generated with CRISPR-Cas9 technology, will be analyzed for transcription factors downstream of LHX1 using RT-qPCR, Hematoxylin-Eosin staining, and immunofluorescence analysis.

To explore extra-renal manifestations of CAKUT, we are also using hPSC-derived retinal-cup organoids, analyzed through immunofluorescence and western blot analysis. Additionally, we are fabricating microfluidic devices using soft-lithography techniques to provide controlled biophysical and biochemical cues, guiding organoid differentiation and cellular composition. Our multidisciplinary approach aims to investigate both renal and extra-renal manifestations of CAKUT, advancing our understanding of the disease and potentially guiding future therapeutic strategies.

POSTER 23 presented by:

NAME: Gal·la Vinyes i Bassols

GROUP: Nanobioengineering

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Bioprinting 3D human neurovascular unit: a high-throughput *in vitro* platform for neurodegenerative diseases modeling and drug screeningGal·la Vinyes i Bassols^{1,3}, Josep Samitier^{1,3}, Anna Lagunas^{2,1}¹ Nanobioengineering group, Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain² CIBER-BBN, ISCIII, Barcelona, Spain³ Department of Electronics and Biomedical engineering, University of Barcelona (UB), Barcelona, Spain

Neurodegenerative diseases (NDDs) are incurable disorders characterized by the progressive structural and functional loss of neurons in specific brain areas^{1,2}. Nowadays, an alarming 80% of NDD drug candidates fail through clinical trials, accounting for the lowest therapeutics approval rate compared to other diseases^{3,4}. This emphasizes the urgent need for reliable NDD models that bridge the bench-to-bedside gap⁵. One major obstacle in developing clinical NDD drugs is the difficult accessibility to the brain, which is controlled by the neurovascular unit (NVU)⁶. The NVU is a cellular network that controls neuroinflammation, maintains bloodbrain barrier (BBB) integrity, and tightly regulates cerebral blood flow⁷.

Over half a century, multiple NVU and BBB models have contributed relevant information to neuroscience research. However, none fully recreate human diseases, and most have limited predictability^{8,9}. Given the significance of NVU models in studying NDDs and the growing interest in developing 3D cell-based models, we aim to engineer a versatile, highly scalable, and cost-effective 3D human *in vitro* platform to mimic NVU in physiological and pathophysiological conditions. Using microvalve-bioprinting and freeform reversible embedding of suspended hydrogels (FRESH) methods, coaxial tubes embedding NVU human cell types will be printed. In this preliminary approximation, self-assembled branching vascular networks have been developed in a ring-shaped configuration. The bioink, containing brain microvascular endothelial cells (BMECs) and an optimized mixture of alginate and fibrinogen, is printed into a viscous cross-linker bath. Rapid cross-linking of the alginate provides temporary structural support to guide fibrin polymerization¹⁰. Afterward, crosslinked alginate is dissolved, and cells are maintained in culture in the fibrin scaffold for 7 days before imaging. In the future, this platform could be used to further characterize the NVU, gain insight into NDDs, and conduct high-throughput drug screening assays. Moreover, the incorporation of patient-derived cells or patient-derived organoids in this 3D model could represent an unprecedented step toward personalized medicine for advanced therapies.

Key words: Neurodegenerative diseases; Neurovascular unit; 3D Bioprinting; Microvessels

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POSTER 24 presented by:

NAME: Anna Vilche

GROUP: Biomaterials for neural regeneration

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Advanced Microphysiological Device for Simulating Traumatic Brain InjuryA. Vilche^{1,2}, P. Chandravanishi¹, A. Noguera^{2,3}, J. A. Ortega^{4,5}, O. Castaño^{2,3,6,7}, Z. Alvarez^{1,6}¹ Biomaterials for Neural Regeneration Group, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain² Department of Electronic and biomedical engineering, University of Barcelona, Barcelona, Spain³ Biomaterials for Regenerative Therapies Group, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain⁴ Department of Pathology and Experimental Therapeutics, Institute of Neurosciences, University of Barcelona, L'Hospitalet de Llobregat, Barcelona, Spain⁵ Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet del Llobregat, Spain⁶ CIBER en Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Madrid, Spain⁷ Institute of Nanoscience and Nanotechnology (IN2UB), University of Barcelona (UB), Barcelona, Spain

Traumatic brain injury (TBI) poses significant challenges due to its complex cellular and molecular mechanisms, which vary with the severity of the trauma.¹ Traditional animal models often fail to accurately recapitulate human physiological responses, limiting their translational relevance. Furthermore, there is a notable lack of humanized *in vitro* platforms capable of recapitulating the dynamic processes involved in traumatic injury. In this study, we present a novel TBI model using a microphysiological device fabricated from PDMS with a thin polymer layer. The device includes two side channels for media flow and a central chamber with an inlet designed to accommodate an impactor, thereby creating controlled contusion lesions. Our innovative model successfully replicates a heterogeneous co-culture that mimics the complexity of brain tissue. We used the impactor to induce controlled damage, allowing precise evaluation of the resulting trauma. We then assessed the damage and investigated potential neuronal repair and regeneration mechanisms within the device. This platform provides a unique and effective method for investigating therapeutic treatments for TBI by providing a reliable and reproducible method for generating and analyzing controlled brain injury. This model is a valuable tool for advancing TBI research and improving the development of targeted therapies.

POSTER 25 presented by:

NAME: Gergo Matajsz

GROUP: Molecular imaging for precision medicine

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

RF Surface Coil Design for High-Throughput Metabolic Imaging using MicrofluidicsGergo Matajsz^[1], David Gomez-Cabeza^[1], Javier Alonso Valdesueiro^[1,2], Irene Marco-Rius^[1]¹ Institute for Bioengineering of Catalonia, 10-12 Baldiri Reixac, 08028 Barcelona² University of Barcelona, Gran Via de les Corts Catalanes, 585, 08007 Barcelona

One of the most pressing challenges of modern healthcare is to determine the stages of complex diseases with a high level of accuracy. Current diagnostic methods (e.g., physical exam and screening mammogram for breast cancer), lack patient specificity and robustness, both of which are detrimental in early diagnostics and in treating the underlying problems. ^[1]. Organ-on-a-chip (OOC) platforms provide a promising low-cost and high-throughput alternative, as observing diseases in a physiologically accurate environment creates ground for better assessment. By combining these microfluidic systems with Hyperpolarized Magnetic Resonance Spectroscopic Imaging (HP-MRSI), we administer labelled compounds (e.g., ¹³C-labelled pyruvic acid) to our biological models located in one of our 8 experimental chambers ^[2]. We polarize our samples with Dynamic Nuclear Polarization (DNP) by cooling to 1.4K, irradiating with microwaves and dissolving them into a buffer solution. When added to our biological models, the labelled compounds go through metabolism, and we acquire decaying signals of low-concentration metabolic products which blend in with the noise under normal circumstances. Therefore, we have a tool to study *in vitro* models that closely mimic the behaviour of tissue conditions. Despite all the advantages outlined above, two crucial challenges remain: efficiently acquiring the quickly decaying hyperpolarized signal and acquiring signals from several samples in parallel.

We use radiofrequency (RF) coils to pick up signal in our 3T MRI scanner. With commercially available coils, maximizing the filling factor of the device is difficult, as they are specifically designed for rodents rather than microfluidic platforms. Birdcage volume coils produce shorter peaks on the chemical shift imaging (CSI) spectrum, indicating weaker signal reception. Moreover, most surface coils designed for rodents are either too small to cover our desired region of interest (ROI) or are only sufficient for 1H-MRSI. Our solution to this problem is maximizing the signal-to-noise ratio (SNR) with a custom-built surface coil, tuned to ¹³C at 3T (32.1 MHz) and matching the size of the microfluidic chambers. The coil will have a separate array element covering each chamber, and due to the thin platform and the negligibly small barrier between the coil and the samples, penetration depth becomes less of a concern. A parallel array will ensure the best signal transmission- and reception in each chamber, without the extra time required for gradient encoding necessary for phased arrays. Upon the completion

of this coil, we expect to see our most robust signal detection system up to date with the highest SNR, showcasing the full potential of HP-MRSI to detect labelled compounds in parallel.

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POSTER 26 presented by:

NAME: Tecla Duran

GROUP: Signal and information processing for sensing systems

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Blind source separation techniques for peak separation in gas chromatography-ion mobility spectrometry data using tensorial decomposition methods*T. Duran¹, L. Fernández^{1,2}, G. Guedes¹, E. Martín¹, A. Pardo^{1,2}, S. Marco^{1,2}*¹ *Signal and Information Processing for Sensing Systems, Institute for Bioengineering of Catalonia (IBEC)*² *Department of Electronics and Biomedical Engineering, Universitat de Barcelona***Introduction**

Gas Chromatography-Ion Mobility technique is a gas phase instrumental technique often used in volatolomics. Despite the high chemical resolution, the complexity of biofluids makes coelution and peak overlapping a common occurrence^[1]. This introduces errors and biases in peak quantification. As a work around for this problem, this study focuses on developing and implementing blind source separation techniques to detect and quantify overlapping peaks in gas chromatography-ion mobility spectrometry (GC-IMS) data using tensor decomposition methods.

The main objective is to develop a software tool that utilizes tensor decomposition to accurately detect peaks in GC-IMS data, even when they are overlapping. This tool is intended to enhance the precision of GC-IMS data analysis, aiding in the identification of volatile compounds in biofluid samples. The functionality is expected to be integrated into the existing R package for GC-IMS data analysis developed by the research group.

The study methodology involves several key steps:

- (i) Generating synthetic data using MATLAB to evaluate various analysis strategies.
- (ii) Developing and implementing blind source separation algorithms^[2] to improve resolution in GC-IMS data.
- (iii) Experimentally validating the implemented techniques using real and simulated samples.
- (iv) Optimizing algorithm parameters to achieve precise and efficient separation of overlapping peaks.
- (v) Comparing the implemented techniques with existing data processing methods in terms of performance and accuracy.

Initial results indicate that the developed software tool can utilize tensor decomposition to detect overlapping peaks in GC-IMS data with high precision. Preliminary experimental validation using synthetic data shows potential improvements in peak detection accuracy. Further validation with real samples is ongoing, and the integration with the existing GC-IMS R package is in progress.

Conclusion

While the project is still ongoing, preliminary findings suggest significant advancements in GC-IMS data analysis capabilities through the introduction of a robust method for detecting overlapping peaks. Upon completion, the developed software tool is expected to provide a valuable resource for researchers and professionals in the field, improving the accuracy of volatile compound identification in biofluid samples and supporting more precise chemical composition analysis.

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POSTER 27 presented by:

NAME: Luis Fernández Romero

GROUP: Signal and information processing for sensing systems

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Application of Multiblock Techniques to Metabolomic and Clinical Data for Predicting Ventilatory Therapies in COVID-19 Patients

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Multiblock techniques are analytical methods designed to handle datasets structured into multiple blocks, each representing a set of variables of a similar nature. These methods aim to model relationships across these blocks, exploring the internal structure of the data to reveal patterns that might be overlooked when analysed individually. This work applies regularized Canonical Correlation Analysis (rCCA), Partial Least Squares (PLS), and multiblock-PLS-DA (DIABLO) techniques to data obtained from Covid-19 patients, focusing on the interactions between two sets of variables: one containing metabolomic measures and the other evaluating various clinical features. Additionally, two different approaches have been proposed and discussed to integrate multiblock analysis into predicting the failure of non-invasive respiratory support in Covid-19 patients. This strategy has facilitated not only the exploration of relationships between blocks but also the identification of similarities and correlations among the relevant variables in a predictive context. Ultimately, multiblock techniques have demonstrated their potential to extract valuable information from the dataset, optimizing and maximizing the classification procedure. This has led to the identification of three key variables—anthranilic acid, octanoic acid, and the malate/succinate ratio—that can successfully predict the failure of non-invasive respiratory support in Covid-19 patients.

POSTER 28 presented by:

NAME: Rishabh Garg

GROUP: Biosensors for bioengineering

INSTITUTION: Birla Institute of Technology

Thread-Based DNA Extraction and Purification with Carbon Dot Fe³⁺ based DNA Biosensor

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Central to the domain of molecular biology resides the foundational process of DNA extraction and purification, a cornerstone underpinning a myriad of pivotal applications. In this research, we introduce a DNA extraction and purification technique leveraging polypropylene (PP) threads. The process commences with robust cell lysis achieved through the vigorous agitation of interwoven PP threads. The friction between the threads facilitates cell lysis especially those having tough cell wall. For purification of DNA, thread-based isotachophoresis was employed which makes the whole process swift and cost-effective. Lysed cell-laden threads were submerged in a trailing electrolyte which separated DNA from other cellular contents. The process was performed with a tailored ITP device. An electric field directs DNA, cell debris, trailing electrolyte, and leading electrolyte toward the anode. Distinct ion migration resulted in DNA concentrating on the PP thread's anode-proximal region. The SYBR green dye is used to visualize DNA as a prominent green zone under blue light. The purified DNA exhibits high purity levels of 1.82 ± 0.1 (A260/A280), making it suitable for various applications aiming at nucleic acid detection.

The development of portable, cost-effective, and straightforward DNA biosensors holds immense importance in various fields, including healthcare, environmental monitoring, and food safety. This study contributes to this goal by presenting a novel approach for synthesizing carbon dots (Cdots) with high quantum yield (QY) and exceptional selectivity for Fe³⁺ ions. Utilizing o-phenylenediamine as a precursor, the study achieved a straightforward and environmentally friendly synthesis method, enabling the efficient detachment of metal ions from the Cdote surface upon introducing pyrophosphate (PPi). The presence of surface hydroxyl and amino groups facilitated specific Fe³⁺ recognition. Employing D-optimal response surface methodology, the study optimized Cdote synthesis parameters, identifying temperature and heating time as critical factors influencing QY. Statistical analysis confirmed the model's reliability, predicting maximum (48.8%) QY values with minimal deviation from experimental results. Characterization studies revealed the amorphous nature of Cdotes through HR-TEM, XRD and FTIR analysis. Furthermore, the proposed LAMP/PPi biosensing technique demonstrated higher sensitivity, specificity, and repeatability, with negligible interference from common anions and efficacy across varying pH levels. The limit of detection (LOD) of 0.079 (± 0.01) μM and 0.1 μM to 2 mM detection range underscore the biosensor's practical utility. This study highlights a promising direction for developing paper-based LAMP/PPi biosensors with potential diagnostics and environmental monitoring applications. Significantly, the biosensing technique is applicable to any DNA amplification method generating pyrophosphate (PPi) as a by-product.

POSTER 29 presented by:

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GROUP: Biomedical signal processing and interpretation

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Measuring High-Resolution Sleep Position and its Variability in Adolescents with Smartphone Accelerometers

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Sleep position affects sleep quality and the risk and severity of different diseases. Classical methods to measure sleep position are complex, expensive, and difficult to use outside the laboratory. Wearables and smartphones can help to address these issues to track sleep position in free-living conditions. Here, we monitor high-resolution sleep position in a large sample of adolescents (N=145) over several nights (1-9) using smartphone accelerometers. This study is part of the Research, Creation and Service program, a Citizen Science project by IBEC and the Education Department of the Government of Catalonia intended for high-school students. We aim to 1) investigate the distribution of sleep positions and position changes in adolescents, 2) study their variability across nights, and 3) propose new measures related to nocturnal body movements.

We developed a new index, the mean sleep angle change per hour, and compared it with classical measures, such as the number of position shifts per hour, the mean time at each position, and the number of periods of immobility. Our results indicate that, overall, participants spent 49% of the time on the side (25% right and 24% left), 36% in supine, and 15% in prone position. This is a higher amount of time in supine position and less in prone than children (3-12 years old), but more time in prone position and less on the side than adults. Moreover, adolescents moved more than adults during sleep according to all measures. There was some variability between nights, but lower than the inter-subject variability. In conclusion, this work systematically analyzes sleep position over several nights in adolescents, a largely unstudied population, and offers a new tool for monitoring high-resolution sleep position and its relationship with sleep quality in a simple and cost-effective way.

POSTER 30 presented by:

NAME: Eva Martin

GROUP: Signal and information processing for sensing systems

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Evaluating the Impact of Respiratory Effort on ICU Survival

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Andrés Parrilla Gómez ²; Joan Ramon Masclans ^{1,2,3}; Santiago Marco ^{5,6}; Eva Martín ⁵

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The study of respiratory effort in mechanically ventilated patients is widely explored to improve medical treatments. This study investigates the relationship between respiratory effort and survival outcomes in 26 ICU patients on mechanical ventilatory support, including 19 survivors and 7 non-survivors. For each patient, three key variables were measured during the ventilatory support in each respiratory cycle: DeltaPes (change in esophageal pressure), PMus (muscle pressure), and DeltaPdi (change in diaphragm pressure). Each cycle was categorized as above normal, within normal, or below normal for each of the three variables. The goal is to determine whether there is a significant difference in the proportion of cycles in these ranges between survivors and non-survivors. Since patients connected to mechanical ventilation for longer periods naturally have more respiratory cycles recorded, there is an unequal number of cycles among patients. To address this imbalance, a weighted least squares (WLS) approach was employed, assigning greater weight to patients with more recorded cycles, thus ensuring that the analysis is not biased by the unequal number of cycles. Preliminary results show a significant difference in the proportion of above-normal and within-normal respiratory cycles between survivors and non-survivors for all three variables: DeltaPes, PMus, and DeltaPdi. Survivors exhibit a higher proportion of cycles within the normal range, whereas non-survivors show a higher proportion of cycles above the normal range, which may be associated with higher levels of respiratory distress. These findings highlight the importance of further studying the respiratory effort to predict outcomes in the ICU. By identifying abnormal respiratory cycles, clinicians can make more accurate prognoses and improve patient outcomes.

POSTER 31 presented by:

NAME: Adriana González

GROUP: Molecular imaging for precision medicine

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Real time biomarker determination of muscular dystrophy type 1Adriana González¹, Silvia Blanco¹, David Gómez Cabeza¹, Juan M. Fernández-Costa², Irene Marco-Rius¹¹ Molecular Imaging for Precision Medicine, IBEC² Biosensors for Bioengineering, IBEC

Myotonic dystrophy type 1 (DM1, OMIM #160900) is an autosomal dominant multi-systemic disorder caused by expansion of a CTG trinucleotide repeat in the 3'-UTR region of DMPK (myotonic dystrophy protein kinase). DM1 is mainly characterized by progressive muscle weakness, atrophy and myotonia, but also prone to ophthalmologic, cardiac, endocrine, neurological, respiratory and cardiovascular problems. It is diagnosed by a set of tests including (1) a genetic test to identify the heterogeneous pathogenic variant and the number of repeats, (2) electromyography, and (3) muscle biopsy. However, muscle biopsy is invasive and has associated risks. Moreover, there is a long delay between treatment and evaluation of its efficacy. Motivated by the lack of DM1 cure and the absence of reliable DM1 biomarkers and early treatment response, we are exploring the use of a non-invasive enhanced nuclear magnetic resonance (NMR) technique, capable of providing sensitivity ~50.000 greater than conventional NMR techniques to determine key biomarkers in real time. As a testbed, we have developed a 2D *in vitro* DM1 model based on the transfection of a CTG960-repeat plasmid in C2C12 myoblast cells. Compared to the control model, the DM1 *in vitro* model showed a significant decrease of bicarbonate production after injection of hyperpolarized [1-¹³C]-pyruvate, being the first time that this molecule signal is detected in a cellular model. Additionally, an increase in cellular death was observed in DM1, together with an impaired myoblast differentiation. Altogether, this data might be explained due to a mitochondrial dysfunction, previously reported in DM1 patients, although further representative data is needed. This DM1 *in vitro* model together with the enhanced NMR technology could set the foundations of a new tool for disease biomarker determination, early diagnosis and a platform for treatment and therapeutic research.

POSTER 32 presented by:

NAME: Consuelo Guardiola

GROUP: -

INSTITUTION: Institute of Microelectronics of Barcelona (IMB-CNM, CSIC)

Novel detectors for advanced radiotherapy at IMB-CNM*Consuelo Guardiola, Carla Riera, Marcio Jiménez, Ángela Henao, Iván López, Celeste Fleita
Institute of Microelectronics of Barcelona (IMB-CNM, CSIC)*

Around 40% of people surviving cancer do so because of radiotherapy. For improving this statistic, treatments based on advanced proton or ultra-high dose rates (FLASH) therapy are allowing a better protection of the organs at risk. Nevertheless, in proton therapy some toxicities have recently been reported, being hypothesized that they can be due to the fact that protons, at the end of their range, deliver higher linear energy transfer (LET) that may generate collateral damages. However, there are no radiation micro-sensors capable of measuring the LET 2D-maps during treatments due to the technology complexity associated. In FLASH therapy we require an accurate dosimetry in ultra-high dose rates, but current silicon-based dosimeters exhibit pulse dose rate dependence in those environments.

To face those challenges, we have created two novel microsensors based on Silicon and Silicon Carbide (SiC) for advanced radiotherapies have been designed and manufactured at the Centro Nacional de Microelectrónica (IMB-CNM, CSIC) in Barcelona (Spain). The former is based on a novel 3D-cylindrical architecture proposed and the second one on particular 4H-SiC p-n diodes, both proposed at IMB-CNM. These microsensors have been successfully tested at two proton therapy clinical centers (ICPO (France) and DCPT (Denmark)), and the Physikalisch-Technische Bundesanstalt PTB (Berlin, Germany). They can be used clinically either as microdosimeters in proton therapy or radiation-hardness dosimeters in ultra-high dose rates in FLASH therapy.

On the one hand, we designed and manufactured a novel silicon 3D-cylindrical microdetectors (25 μm diameter, 20 μm depth, and 200 μm pitch) ^[1-4]. In particular, the present work shows four new multi-array configurations of these sensors (microdosimeters), first of its kind, enable of quantifying the LET 2D-maps in clinical conditions and covering a wide range of resolutions, namely: (i) a 11x11 array covering a 2 mmx2 mm radiation sensitive area ^[1,2] and (ii) a linear array of 3 x 3 microdetectors with a total surface of 0.4 mmx12 cm ^[3]; (iii) a 5x25 pixel configuration covering an area of 1.9 cm x 0.1 cm and (iv) a 1x10 strip layout of 5.1 cm x 0.1 cm ^[4]. The microdosimetry 2D-maps were obtained with a spatial resolution of 200 μm , the highest achieved so far at different positions of the Bragg curve by using a water-equivalent phantom (Fig. 1, left). We will show the results of both sets of experiments. All the experimental results were crosschecked with Monte Carlo simulations and also compared to literature results. To the best of our knowledge, these are the largest radiation sensitive surface covered with microdosimeters by now.

On the other hand, silicon carbide (SiC) diodes have been developed for dosimetry for ultra-high dose-per-pulse (DPP) radiation at FLASH radiotherapy (Fig.2, left). The linearity of the diode response was investigated and it was independent both of DPP and of pulse dose rate up to at least 11 Gy per pulse and 6 MGy s, respectively, with tolerable deviation for relative dosimetry (<5%). When measuring the percentage depth dose under ultra-high dose per pulse conditions, the SiC diode performed comparably well to a reference diamond dosimeter. The sensitivity reduction after 100 kGy accumulated dose was <2% of 20 MeV electrons ^[5].

We have demonstrated for first time that the new microsensors designed and manufactured at IMB-CNM may obtain dosimetry distributions in both advanced proton and FLASH therapy, which could contribute to the optimization of patient treatments.

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POSTER 33 presented by:

NAME: Gema Guedes de la Cruz

GROUP: Signal and information processing for sensing systems

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Searching for a rapid tool to identify high quality microbiota donations for specific faecal microbiota transplants

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The gut microbiota of healthy individuals can be considered an organ with an immune function. A large number of clinical trials highlight the potential clinical impact on health through the transplant of healthy faecal microbiota (TFM).¹

Gut microbiota is unique for each individual and influenced by a wide range of factors, including diet, microbiota exposure during the first years of life, hygiene conditions, pollution, medication, socioeconomic status and other environmental factors.² However, it has been established that a high concentration of short-chain fatty acids (SCFAs), known as a small group of immunomodulatory metabolites, is a good biomarker of healthy microbiota.³

Current methods to measure SCFAs are time- and resource-consuming, which limits the ability to screen a large number (hundreds to thousands) of donor samples. Here, we propose the development of rapid screening tool that allow the identification of high-quality samples in microbiota biobanks to select suitable samples for advanced clinical indications, such as FMT prior to immunotherapy for cancer treatment.

In collaboration with the Hospital Clinic of Barcelona, we are investigating both the use of infrared spectroscopy and ion mobility spectrometry as a rapid tool to identify and classify high quality microbiota. Although these two techniques yield immediate analytical results, statistical data processing is required to aid in the classification process.

In this contribution, we will discuss advances in the use of both techniques for the classification and identification of high-quality microbiota.

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POSTER 34 presented by:

NAME: Manuel Lozano García

GROUP: Biomedical signal processing and interpretation

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Respiratory Sound Intensity as a Noninvasive Acoustic Biomarker in COPD

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Chronic obstructive pulmonary disease (COPD) is a common respiratory disease and a leading cause of death worldwide. Due to the clinical heterogeneity of COPD and the low specificity of the spirometric tests currently used for diagnosing COPD, it is often under-diagnosed. Improving the diagnosis and monitoring of COPD requires further research into new biomarkers. Respiratory sound (RS) intensity, quantified as the amplitude of RS signals, is closely related to respiratory airflow, and has been proposed as an indirect measurement of the airflow entering the lungs ^[1]. This study aims to explore the potential use of RS intensity, in comparison to respiratory flow, as a noninvasive acoustic biomarker in COPD ^[2].

Respiratory flow and RS signals were recorded in 15 healthy controls, 7 mild COPD patients (forced expiratory volume in 1 second (FEV1) \geq 50% predicted) (COPD $>$ 50), and 5 severe COPD patients (FEV1 $<$ 50% predicted) (COPD50 (305.7 (260.0-353.8)) and COPD 0.90) with peak flow for all four RS channels. When compared at normalized flow levels, RS intensity was higher in healthy subjects than in COPD patients at medium/high flow levels, reflecting airflow limitation of COPD patients when respiratory demand increases. However, baseline RS intensity was slightly higher in COPD50 patients, reflecting the higher effort performed by COPD $<$ 50 patients to achieve a proper ventilation during normal breathing due to their intrinsic respiratory mechanical load. When compared at similar peak flow values, higher RS intensity was observed in COPD50 patients and healthy subjects, reflecting the higher flow velocity caused by the higher degree of airway obstruction of COPD $<$ 50 patients.

These results show that there are differences in RS intensity generated by healthy subjects and COPD patients, especially at medium/high inspiratory flows, and over the posterior right lung. These results therefore demonstrate that RS intensity is sensitive to airflow limitation caused by altered respiratory mechanics in COPD, and could be used to derive noninvasive acoustic biomarkers to improve the diagnosis and long-term monitoring of COPD patients.

Research supported by Generalitat de Catalunya (CERCA Program, GRC 2021 SGR 01390), Agencia Estatal de Investigación, Ministerio de Ciencia, Innovación y Universidades, Gobierno de España and European Regional Development Fund (PID2021-126455OB-I00), Centro de Investigación Biomédica en Red Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), and European Respiratory Society (ERS LTRF 2015-5185).

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POSTER 35 presented by:

NAME: Martín Ruíz Gutiérrez

GROUP: Biosensors for bioengineering

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Integrated Organ-on-Chip Platform with PINP Plasmonic Biosensor for Fibrosis Monitoring in Duchenne Muscular Dystrophy

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Muscular dystrophies encompass a wide range of debilitating diseases characterized by progressive muscle weakness and atrophy. In affected individuals, skeletal muscles undergo progressive loss of muscle fibers, replaced by fibrotic tissue that prevents muscle regeneration and limits the response to therapeutic interventions. Here, we present an innovative approach to monitor fibrosis *in vitro* using 3D cultures of fibro adipogenic progenitor cells (FAPs) coupled with optical sensors. Initially, we characterized collagen accumulation in 3D FAP cultures derived from Duchenne Muscular Dystrophy (DMD). While conventional methods like ELISA and soluble collagen assays (SIRCOL) did not detect increased collagen-I in the culture supernatants, we identified a significant increase of the N-terminal propeptide of type 1 collagen (PINP), a marker of collagen-I fiber formation. To monitor fibrosis progression, we developed a plasmonic sensor specifically targeting PINP. This surface plasmon resonance-based optical sensor enables label-free, real-time detection of PINP peptide. Integration of this platform involved incorporating FAP 3D cultures into a microfluidic organ-on-chip device linked to nanoplasmonic biosensors for PINP detection. This biosensing platform facilitates the continuous monitoring of collagen synthesis by FAPs, offering a valuable tool for screening potential anti-fibrotic therapies and new treatments for muscular dystrophies.

POSTER 36 presented by:

NAME: Daniel Romero Perez

GROUP: Biomedical signal processing and interpretation

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Multivariable Regression Model to Estimate Tidal Volume for Different Respiratory Patterns*Daniel Romero, Jordi Sola-Soler, Leon Balchin, Arantxa Mas, Manuel Lujan, Melinda R Popoviciu, Beatriz F Giraldo**Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain.*

Respiratory patterns present great variability, both in healthy subjects and in patients with different diseases and forms of nasal, oral, superficial or deep breathing. The analysis of this variability depends, among others, on the device used to record the signals that describe these patterns. In this study, we propose multivariable regression models to estimate tidal volume (V_T) considering different breathing patterns. Twenty-three healthy volunteers underwent continuous multisensor recordings considering different modes of breathing. Respiratory flow and volume signals were recorded with a pneumotachograph and thoracic and abdominal respiratory inductive plethysmographic bands. Several respiratory parameters were extracted from the volume signals, such as inspiratory and expiratory areas ($Area_{ins}$, $Area_{exp}$), maximum volume relative to the cycle start and end (VT_{ins} , VT_{exp}), inspiratory and expiratory time (T_{ins} , T_{exp}), cycle duration (T_{tot}), and normalized parameters of clinical interest. The parameters with the greatest individual predictive power were combined using multivariable models to estimate V_T . Their performance was quantified in terms of determination coefficient (R^2), relative error (E_R) and interquartile range (IQR). Using only three parameters, the results obtained for the thoracic band (VT_{exp} , T_{tot} , $Area_{exp}$) were better than those obtained from the abdominal band (VT_{exp} , T_{ins} , $Area_{ins}$) with $R^2 = 0.94$ (IQR: 0.07); $E_R = 6.99$ (IQR: 6.12) vs $R^2 = 0.91$ (IQR: 0.09), $E_R = 8.70$ (IQR: 4.62). Overall performance increased to $R^2 = 0.97$ (IQR: 0.02) and $E_R = 4.60$ (IQR: 3.68) when parameters from the different bands were combined, further improving when was applied to segments with different inspiration–expiration patterns. In particular, the nose–nose $E_R = 1.39$ (IQR: 0.73), nose–mouth $E_R = 2.11$ (IQR: 1.23) and mouth–mouth $E_R = 2.29$ (IQR: 1.44) patterns showed the best results compared to those obtained for basal, shallow and deep breathing. Clinical relevance—Respiratory pattern variability can be described using multivariable regression model for tidal volume.

POSTER 37 presented by:

NAME: David Gomez-Cabeza

GROUP: Molecular imaging for precision medicine

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Parallel Metabolic Imaging Using MRI and Microfluidics for Personalised Medicine

David Gomez-Cabeza¹, Marc Azagra¹, Alba Herrero-Gomez¹, Lluís Mangas-Florencio^{1,2}, James Eills¹,
Alejandro Portela², Gergo Matajcz¹, Irene Marco-Rius¹

¹ Molecular Imaging for Precision Medicine, Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain.

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The high complexity of metabolic diseases drives researchers to seek holistic, yet personalised, strategies to treat them. Organs-on-a-chip (OoC) are promising candidates to develop complex patient-derived biological models to study such diseases and potential treatments in a parallel manner, with the end goal of being translatable to patients. Hence, OoC allows us to model organs and tissues to study concrete diseases, aiming at circumventing the need for animal trials and adding advantages to the process (e.g., lower costs or models derived from patient organs for personalised medicine). Yet, to truthfully evaluate metabolism to infer cellular or organ state (e.g., healthy or diseased), we need fast, non-invasive and non-destructive methodologies inaccessible to many research groups.

In this work, we combine microfluidics and hyperpolarised magnetic resonance spectroscopic imaging (HP-MRSI) to non-destructively study cell metabolism in real-time and establish a platform for parallel studies of disease treatments. We leveraged ¹³C labelled sugars (i.e. [1-¹³C]pyruvate) to track highly relevant metabolism (conversion into [1-¹³C]lactate) in HepG2 cells in suspension. To surpass the sensitivity limitations and significantly reduce the acquisition times of these fast processes, we polarised the substrate with a dDNP HyperSense (Oxford Instruments). To emphasise the parallelisable potential of combining microfluidics with HP-MRSI, we evaluated 8 different conditions by using Chemical Shift Imaging (CSI) with our 3T MRI scanner (BioSpec, Bruker), shown in Fig. 1. The base sample was cells in EMEM media (1% P S, 10% FBS). We supplemented two samples with Glucose (Glu, 25mM) and Glutamine (Gln, 4mM) and two samples with NADH (33mM) (reaction cofactor). We also lysed three samples with RIPA buffer. Finally, we left two samples as positive (commercial enzyme) and negative (only media) controls.

We successfully parallelised the acquisition of metabolic imaging, understanding the role of rich media, cell lysis and extracellular NADH in converting pyruvate to lactate. With this type of voxel spectroscopic metabolic imaging provided by CSI, we observed three different phenomena in a single experiment. First, we observed the increase of lactate production in a rich media supplemented with Glu and Gln due to an increase in glycolytic activity, as previously reported in literature. Second, extracellular NADH

helps adjust the redox balance, in alive (indirectly) and lysed (directly) cells, resulting in an increase in lactate production. Third, by lysing cells (and adding NADH), the metabolism of lactate was significantly increased thanks to avoiding the main limiting factor of the reaction, the transport of pyruvate into the cell tanks to the MCT1 transporter. Yet, despite the advantages of the strategy, better detection coils with large areas (we employed a volume coil) and better k-space sampling with low pulsing are required to improve the resolution and aim for temporal imaging. Nonetheless, our results are the first to show such level of parallelisation with high spatial resolution for cellular studies using microfluidics and HP-MRSI, contributing to incorporating hyperpolarised NMR into the field of precision medicine.

POSTER 38 presented by:

NAME: Mamatha Nijaguna

GROUP: Cellular and molecular mechanobiology

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Inhibiting mechanotransduction as a novel approach for oncology therapyMamatha Bangalore Nijaguna¹, Ignacio Viciano Gonzalo^{1*}, Borja Mateos³, Anabel-Lise Le Roux¹,Evelyn Coderch Bifet¹, Xavier Salvatella², Pere Roca-Cusachs Souler^{1,2}¹ Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain,² Universitat de Barcelona, Barcelona, Spain, ³Institute for Research in Biomedicine (IRB), Barcelona, Spain,

*Former researcher at IBEC

Increased tissue stiffness is a prevalent feature in most solid tumors. Normally, tissues and cells sense the surrounding stiffness and transduce this mechanical signal into biological activity through the process of mechanotransduction. This process is tightly regulated to maintain tissue homeostasis. In the case of solid tumors, increased tissue stiffness enhances mechanotransduction, promoting tumor progression. Our lab has identified a crucial interaction between a target protein and its binding partner, which is vital for mechanotransduction. The interaction is triggered by force-induced target protein unfolding, which only occurs in stiff tissue, initiating mechanotransduction. We thus propose a novel approach targeting mechanotransduction by inhibiting the stiffness-induced unfolding of the target protein. This approach is unconventional and has therapeutic application in several cancer types and beyond cancer.

Towards this goal, we have developed a thermal shift assay for our target and conducted High-throughput Screening (HTS) of ~5,000 molecules. This approach resulted in 6 confirmed hit compounds that thermally stabilize the target protein; of which, for 4 compounds, the NMR data suggested specific binding to the target protein. Beside HTS, we also used structure-based virtual screening to identify hits, which resulted in one hit molecule with confirmed target protein binding by NMR. We aim to valorize these hits for oncology therapy application. As an alternate approach, we designed both poly glutamine modified, and staple peptides based on the bonafide binding partner to our target. Both these peptides showed improved alpha helicity compared to WT peptide and shows specific binding to target protein by NMR. However, only the staple peptide thermally stabilized the target protein with improved binding affinity compared to WT. Hence it is a promising hit to validate in further studies along with small molecule hits. At present, we have work ongoing to validate the target protein specific hits in relevant *in vitro* and cellular assays to delineate the mechanism of action. Finally, we plan to evaluate the efficacy of lead molecules *in vivo* mouse breast cancer model system.

The outcome of this study will be a first-in-class mechanoinhibitor with therapeutic applications in oncology and various other pathological conditions. This research not only holds promise for developing innovative cancer therapies but also provides tool compounds that contributes to advancing our understanding of fundamental mechanobiology processes.

POSTER 39 presented by:

NAME: Pau Guíllamat

GROUP: Integrative cell and tissue dynamics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Guidance of cellular nematics into self-shaping active surfacesGuíllamat, Pau ¹, Mirza, Waleed ², K. Bal, Pradeep ³, Gómez-González, Manuel ¹, Arroyo, Marino ³, Trepát, Xavier ¹¹ Institute for Bioengineering of Catalonia (IBEC), Barcelona, Catalunya.² European Molecular Biology Laboratory (EMBL), Barcelona, Catalunya.³ LaCàN, Universitat Politècnica de Catalunya (UPC), Barcelona, Catalunya

The ability to harness the contractility from cells and tissues has opened the door to engineering synthetic living materials with emergent mechanical properties ^[1–3]. Despite recent efforts for leveraging cytoskeleton-driven forces in biohybrid devices ^[3], achieving efficient control of the cells' mechanics remains still a remarkable challenge. Here, by drawing inspiration from tissue morphogenesis ^[4], we program contractile cellular sheets to generate specific shape transformations driven by supracellular force patterns fueled by cytoskeletal contractility. In particular, we use cellular nematics – tissues composed of elongated cells – where the organization of subcellular forces is dominated by cell orientation in nematically-ordered domains, and the presence of topological defects, areas where order is lost ^[5]. By directly controlling cellular orientation and topological defects, which can provide essential mechanical cues for tissue remodeling ^[6–9], we organize millimeter-scale cell monolayers with programmed multicellular tension patterns that can be released via out-of-plane deformations to form reproducible three-dimensional shapes. This strategy will not only enable the mapping of morphogenetic events within living tissues, but also potentially lead to applications in tissue engineering ^[10] and soft robotics ^[3].

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POSTER 40 presented by:

NAME: Annalisa Calò

GROUP: Nanoscale bioelectrical characterization

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Mechanical phenotyping of lung cancer CAFs*Annalisa Calò**Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST),
Barcelona, Spain.*

The tumour microenvironment (TME) is gaining progressive attention for its physical-chemical properties, favouring cancer spreading and therapeutic resistance. Among TME components, cancer-associated fibroblasts (CAFs) are critical players, whose features are not fully characterized (1). In our work, we use AFM-based mechanical mapping to study CAFs from two distinct subtypes of non-small cell lung cancer (NSCLC). Using optimized measurement protocols (2), we achieved a robust CAFs mechanical characterization (Y, adhesion force) across millimetre-size cell layers. The obtained results show significant differences among the two patients' groups, even in morphologically similar CAFs cells. These results show the potential of the AFM technique for highly sensitive functional characterization of primary cells, thus making it an interesting tool for clinical applications.

POSTER 41 presented by:

NAME: Clément Hallopeau

GROUP: Integrative cell and tissue dynamics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Mechanisms of mechanical compartmentalisation in intestinal organoids

Clément Hallopeau ¹, Gerardo Ceadá ¹, Anghara Menéndez ¹, Sergio Palomo ², Carlos Perez Gonzalez ³, Marija Matejčić ¹, Eduard Batlle ², Xavier Trepac ¹

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Monolayers of intestinal organoids recapitulate the functional compartmentalisation seen in-vivo. Crypt-like regions host stem cells, Paneth cells and transit amplifying cells, whereas villus-like regions contain differentiated cells. Measurements of traction forces in these organoids have established that stem cells push the underlying substrate while the transit-amplifying cells pull it, defining clear mechanical and functional compartments (Pérez-González, Ceadá et al, Nat Cell Bio, 2021). Crypt compartmentalisation is attributed to a gradient in Eph/ephrin signaling, but how this gradient is linked to the mechanical pattern is unknown. To address this question, we studied the mechanical and functional compartmentalisation in organoids derived from mice lacking EphB2 and EphB3 (EphB2^{-/-}, EphB3^{-/-}). We found that, unlike in wild type organoids (WT), crypts of EphB2^{-/-}-EphB3^{-/-} organoids (KO) expand at the expense of the villus-like region. This phenotype is associated to an increased proliferation of the KO crypts. In mechanical terms, the 3D traction pattern of the KO crypts is qualitatively similar to the WT, but forces have a decreased amplitude, suggesting a decreased tension around the KO crypts. Taken together, these data establish a link between the mechanical features and the size homeostasis of the functional compartments, governed by Eph/ephrin signaling in intestinal organoids.

POSTER 42 presented by:

NAME: Guillermo Martínez Ara

GROUP: Integrative cell and tissue dynamics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

An optogenetic toolset to understand and control epithelial mechanical balanceGuillermo Martínez Ara¹, Emmanuel Spanoudakis¹, Xavier Trepât Guixer^{1,2,3}.¹ Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute for Science and Technology (BIST), Barcelona, Spain² Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Barcelona, Spain³ Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

Cells form tissue structures through the interaction of mechanical forces¹. Synthetic biology proposes the control of such forces to improve our understanding of how tissue structures arise². In addition, optogenetics has opened the possibility of gaining spatio-temporal control of mechanical forces with light³. These approaches have proven to be useful for the study of epithelial morphogenesis^{4,5}. However, the experimental control achieved doesn't account yet for all the forces proposed in physical models of tissue morphogenesis. Several theoretical studies propose an epithelial mechanical balance between apical, lateral, and basal contractility⁶. In this project, we make use of optogenetic and synthetic approaches to gain control over this set of forces (apical, basal, and lateral contractility) to test whether they are sufficient to understand and control the shape of different epithelial cell types.

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POSTER 43 presented by:

NAME: Aina Albajar Sigalés

GROUP: Cellular and molecular mechanobiology

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Studying the mechanical regulation of nucleocytoplasmic transport using Single Molecule Tracking

Albajar-Sigalés, Aina ^{1,2}; Le Roux, Anabel-Lise ¹; Venturini, Valeria ¹; Pons, Roger ³; García-Parajo, María F. ^{3,4}; Pujals, Sílvia ⁵; Beedle, Amy E.M. ^{1,6}; Roca-Cusachs, Pere ^{1,2}

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Cellular function relies on the precise regulation of macromolecular transport between the cytoplasm and the nucleus, a process governed by Nuclear Pore Complexes (NPCs). While traditional understanding held that transport through NPCs was mainly dictated by the physicochemical properties of the transported molecules, recent research has revealed an additional layer of regulation driven by mechanical forces. Whether applied directly or transmitted through the cytoskeleton, mechanical signals have the capacity to deform the nucleus and, consequently, alter the conformation of NPCs, increasing their diameter. This changes nucleocytoplasmic transport rates and impacts the localisation of a variety of signalling molecules. However, the cellular components responsible for force transmission to NPCs as well as the spatial distribution of this effect throughout the nuclear envelope, remain unknown. Here, we aim to answer these questions by investigating whether isolated nuclei, a minimal system depleted of all the cell's cytoplasmic machinery, still exhibit mechanosensitive nucleocytoplasmic transport. To do so, we apply mechanical force by confining isolated nuclei to a certain micrometre height and, simultaneously, measure nucleocytoplasmic transport rates by tracking individual dextran molecules translocating through NPCs. We believe our study will help unveiling the spatial and molecular determinants behind the mechanical regulation of nucleocytoplasmic transport.

POSTER 44 presented by:

NAME: Miguel González Martín

GROUP: Cellular and molecular mechanobiology

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Designing mechanosensible molecules for the mechanical control of cellular transcription.

Miguel González-Martín^{1,2}, Juan-Martin Portilla³, Marc Molina Jordán^{1,2}, Ignasi Granero-Moya^{1,2}, Dimitrije Ivančić³, Ion Andreu^{4,5}, Marc Güell³, Pere Roca-Cusachs^{1,2}.

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Cells sense mechanical signals in the process of mechanotransduction, activating pathways that govern cell behavior. However, it remains a challenge to engineer mechanotransduction pathways in a controllable and predictable manner. Here we aim to engineer a synthetic mechanosensitive transcription factor (msTTA). To this end, we exploit the force induced changes in nuclear transport, linking nuclear mechanical perturbations to gene expression. To do so, we are mechanically tuning the passive and facilitated transport properties of the synthetic msTTA. Through this we aim to recapitulate the localization behavior of endogenous mechanosensitive proteins such as YAP or Twist, but with a synthetic factor that activates genes of choice in a controlled way. Optimizing our reporter cells, we have set up a novel screening platform with substrates of different rigidity, from which we expect to identify highly mechanosensitive TF candidates that function in a tunable manner, as well as to elucidate which features make a transcription factor mechanosensitive. Overall, we expect to unlock precise transcriptional control through mechanical forces, and a state-of-the-art directed evolution platform for msTTAs. With the simplicity of this engineered regulatory module, we expect to describe the minimal elements of mechano-regulation of gene expression, as well as enabling the use of mechanotransduction in gene circuits control. This will open the field to use mechanotransduction approaches for complex synthetic biology applications.

POSTER 45 presented by:

NAME: Mariana Azevedo Gonzalez Oliva

GROUP: Microenvironments for Medicine

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Piezo1 is a mechanosensor of matrix viscoelasticity

Mariana A. G. Oliva¹, Giuseppe Ciccone¹, Jiajun Luo¹, Jonah L. Voigt^{1,5,6}, Patrizia Roman², Oana Dobre¹, Sirio Dupont², Massimo Vassalli², Manuel Salmeron-Sanchez^{1,3,4*}

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In the last 30 years, it has been shown that cells exert and perceive mechanical forces as part of physiological (e.g., tissue formation) and pathological (e.g., cancer progression) processes; leading to what is now known as the field of mechanobiology. Mechanosensitive ion channels have emerged as fundamental proteins in sensing extracellular matrix (ECM) mechanics. Among those, Piezo1 has been proposed as a key mechanosensor in cells. However, whether and how Piezo1 senses time-dependent ECM mechanical properties (i.e., viscoelasticity) remains unknown. To address this question, we combined an immortalised mesenchymal stem cell (MSC) line with adjustable Piezo1 expression with soft (400 Pa) and stiff (25 kPa) viscoelastic hydrogels with independently tuneable Young's modulus and stress relaxation. We demonstrate that Piezo1 is a mechanosensor of viscoelasticity in soft ECMs, consistent with the molecular clutch model. By performing RNA sequencing (RNA-seq), we identified the transcriptomic phenotype of MSCs response to matrix viscoelasticity and Piezo1 activity, highlighting gene signatures that drive MSCs mechanobiology in soft and stiff viscoelastic hydrogels.

POSTER 46 presented by:

NAME: Ona Baguer Colomer

GROUP: Cellular and molecular mechanobiology

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Role of nuclear mechanics in the regulation of EMT in pancreatic cancer cells*Baguer, Ona^{1,2}, Faure, Laura M.¹, Zagorac, Sladjana³, Chesnokov, Mikhail³, Real, Francisco X.^{3,4}, Roca-Cusachs, Pere^{1,2}*¹ *Institute for Bioengineering of Catalonia (IBEC)*² *University of Barcelona (UB)*³ *Spanish National Cancer Research Center (CNIO)*⁴ *Pompeu Fabra University (UPF)*

Pancreatic cancer is associated with a strong desmoplastic reaction, leading to a stiffening of the tissue that is known to induce the onset of the epithelial-to-mesenchymal transition (EMT). Both tissue stiffening and EMT are strongly associated with changes in cell and nuclear shape. In turn, nuclear shape changes (i.e., nuclear deformations) are known to trigger nuclear mechanotransduction events that can induce signalling. This suggests that tissue stiffening, EMT, and nuclear mechanotransduction could be related, but if and how this occurs is unknown. To address this issue, we combine the use of hydrogels of different rigidities, genetic tools, and EMT-inducing biochemical cues (TGF β), providing a system that allows us to control the force exerted on the nucleus, nuclear mechanics, and EMT. By tuning these three elements and characterising cell behaviour, our preliminary data suggest that nuclear mechanics and deformation regulates pancreatic cancer cell responses to both stiffness and TGF β in a similar manner, potentially unveiling a conserved underlying mechanotransduction mechanism. In further work, we expect to untangle the role of nuclear mechanics in EMT, within the context of pancreatic ductal adenocarcinoma.

Keywords: nuclear mechanics, EMT, nuclear lamina, pancreatic cancer.

POSTER 47 presented by:

NAME: Giuseppe Ciccone

GROUP: Microenvironments for Medicine

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Matrix viscoelasticity controls epithelial cell mechanobiology through dimensionality*Giuseppe Ciccone^{1,2,3}, Mariana Azevedo Gonzalez Oliva^{1,3}, Marie Versaemel², Massimo Vassalli³, Marco Cantini³, Manuel Salmeron-Sanchez^{* 1,3}, Sylvain Gabriele^{* 2}**¹ Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain**² Mechanobiology & Biomaterials group, CIRMAP, University of Mons, Mons, Belgium**³ Centre for the Cellular Microenvironment, University of Glasgow, Glasgow, UK*

In recent years, matrix viscoelasticity has emerged as a potent regulator of fundamental cellular processes and has been implicated in promoting cancer progression. Alongside viscoelasticity, additional ECM cues have been shown to influence migration decision-making of cancer cells, and spatial confinement is now considered as a potential regulator of metastasis. However, our understanding of these complex processes predominantly relies on purely elastic hydrogels, and the exact relationship between matrix viscoelasticity and spatial confinement in driving epithelial cell mechanotransduction and migration during cancer progression remains unclear. Here, we systematically investigated the interplay between matrix stiffness, viscoelasticity and spatial confinement by engineering soft (~0.3 kPa) and stiff (~3 kPa) polyacrylamide hydrogels with varying degrees of viscous dissipation, mirroring the mechanical properties of healthy and tumoral conditions in breast tissue. We observed that viscoelasticity modulates cell spreading, focal adhesions and YAP nuclear import in opposite directions on soft and stiff substrates. Strikingly, viscoelasticity enhances migration speed and persistence on soft substrates, while impeding them on stiff substrates via actin retrograde flow regulation. Combining soft micropatterning with viscoelastic hydrogels, we also show that spatial confinement restricts cell migration on soft matrices regardless of matrix viscoelasticity and promotes migration on stiff matrices in a viscoelasticity-dependent fashion. Our findings establish substrate viscoelasticity as a key regulator of epithelial cell functions and unravel the role of the matrix dimensionality in this process.

POSTER 48 presented by:

NAME: Miquel Bosch

GROUP: Integrative cell and tissue dynamics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Force transmission in embryonic-like epithelia*Miquel Bosch-Padrós, Guillermo Martínez-Ara, Miki Ebisuya, Xavier Trepât*¹ *Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain (Miquel, Guillermo, Xavier)*² *European Molecular Biology Laboratory (EMBL), Barcelona, Spain (Miki)*³ *RIKEN Center for Biosystems Dynamics Research (RIKEN BDR), Kobe, Japan (Miki)*⁴ *Cluster of Excellence Physics of Life, TU Dresden, Dresden, Germany (Miki)*⁵ *The Barcelona Institute for Science and Technology (BIST), Barcelona, Spain (Xavier)*⁶ *Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y nanomedicina (CIBER-BBN), Barcelona, Spain (Xavier)*⁷ *Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain (Xavier)*

The function a tissue performs is dictated not only by containing the correct cell types, but also but also by controlling the shape they adopt. Sculpting the correct structures requires precise force generation at the cellular level and coordinated force transmission to the surrounding tissue. Among all mechanisms to undergo morphogenesis, apical constriction stands out as a relevant process and is conserved across the animal kingdom. Nonetheless, current knowledge lacks measurements of the forces involved in it. By triggering this mechanism optogenetically in human iPSCs, we report quantitative measurements of force generation and tissue deformation during apical constriction, which we find to be extremely efficient at transmitting force due to a mechanical decoupling of its apical and basal layers. Moreover, material properties of this embryonic-like tissue can be inferred, which have an unknown yet relevant role in reshaping during human development. Taken together, our results describe quantitatively the mechanical behavior of a pluripotent tissue and shines light on the mechanically inaccessible world of human morphogenesis.

POSTER 49 presented by:

NAME: Zarina Nauryzgaliyeva

GROUP: Pluripotency for organ regeneration

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Dissecting early nephron patterning and segmentation in kidney organoids derived from hPSCsZarina Nauryzgaliyeva^{1,2}, Miquel Bosch², Andres Marco¹, Idoia Lucia Selfa¹, Xavier Trepac^{2,5}, Elena Garreta¹, Nuria Montserrat^{1,4,5}¹ Pluripotency for Organ Regeneration group, Institute for Bioengineering of Catalonia (IBEC), the Barcelona Institute of Technology (BIST), C Baldri Reixac 15-21, 08028 Barcelona, Spain² Institute for Bioengineering of Catalonia, Barcelona 08028, Spain. ³ University of Barcelona, Barcelona 08028, Spain⁴ University of Barcelona, Barcelona, 08028, Spain⁵ Networking Biomedical Research Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain.⁶ Catalan Institute for Research and Advanced Studies (ICREA), 08010 Barcelona, Spain**Introduction.**

Organ formation during development is a complex, yet beautifully orchestrated process. Embryonic cells have a unique capacity to self-organize within the forming tissue, where morphogenetic movements facilitate tissue organization and subsequent organ formation. In kidney organogenesis, the mature organ arises from crucial reciprocal interactions between the ureteric bud (UB) and metanephric mesenchyme (MM), which give rise to the collecting duct and nephron, respectively. The development of mature nephrons during kidney organogenesis is a dynamic process so far studied via *in vivo* models. Accumulative findings in mice have shown that the MM undergoes mesenchymal to epithelial transition (MET), giving rise to epithelial renal vesicles (RVs) that further undergo structural changes and shift towards comma-shaped and s-shaped bodies (CSBs/SSBs), which eventually develop into nephron-like structures. Those studies have helped identify Wnt b-catenin and Notch signaling pathways as key players in nephron patterning and segmentation (proximal, medial, and distal segments).

At the same time, tissue morphogenesis is largely a biomechanical process, resulting from constant movements of cells, changes in forms of developing segments, and forces generated therein. The biomechanical dynamics occurring during RV emergence and further nephron patterning are yet to be explored in the human context in real-time. Whether these processes are interconnected with mechanical signals remains an open question in the field. The answer may have an important impact on understanding nephron formation, and conversely, disease-related phenotypes due to mutations in genes orchestrating RV patterning and segmentation, as seen in congenital defects of the kidney and the urinary tract (CAKUT disease).

Objective.

Here, we aim to use human pluripotent stem cell (hPSCs) derived kidney organoids of wild type and KO backgrounds (for CAKUT-related genes) to gain fundamental understanding of early nephron patterning and segmentation by mapping force transmission between cells and their extracellular matrix (ECM) and evaluating their co-evolution during renal fate specification and differentiation.

Methodology.

hPSCs are guided towards the renal fate on compliant PDMS substrates with controlled rigidities (mimicking embryonic microenvironment) and geometries in a 2D culture system. PDMS substrates of 3 kPa (soft) are generated by adapting the compositional ratio of PDMS components, photopatterned into circular, square, and triangular geometries, and are further functionalized and decorated with fibronectin. Using this system, we have started to spatiotemporally characterize early steps of nephrogenesis by immunofluorescence and confocal analysis, time-lapse imaging, and traction force microscopy (TFM). These analyses are conducted during RV emergence before proximal-distal RV polarization and formation of the nephron-like segments in kidney progenitors of wild-type and CAKUT-mimicking backgrounds.

Conclusion. The current techniques permit quantitative and qualitative observations of multicellular behaviors at key stages of 2D renal differentiation. Furthermore, this system allows us to spatiotemporally map overall forces and evaluate their evolution throughout renal fate specification with the final aim of decoupling mechano-related processes sustaining nephron formation from classical biochemical signaling.

POSTER 50 presented by:

NAME: Isabela Corina Fortunato

GROUP: Integrative cell and tissue dynamics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Following and unfollowing haptotactic gradients

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Directed cell migration along gradients of extracellular matrix (ECM) density –a process called haptotaxis– plays a central role in morphogenesis, the immune response, and cancer invasion. It is commonly assumed that cells respond to these gradients by migrating directionally towards the regions of highest ligand density. In contrast with this view, here we show that the integration of ECM gradient sensing and persistent polarity dynamics can give rise to non-trivial migration trajectories, including migration against the gradient and persistent circles. We generated symmetric gradients of fibronectin density confined to rectangular areas of different width. As expected, upon adhering to these patterns, cells polarized and migrated robustly towards the direction of the highest protein density. However, after reaching the maximal density, cells exhibited different migration patterns depending on the gradient width. On confined 1D gradients, cells failed to repolarize and continued to migrate persistently against the fibronectin gradient. By contrast, on wide gradients, they made a 90° turn and migrated along the ridge defined by the maximal fibronectin density. For intermediate widths, non-trivial trajectories such as circles emerged. Overall, our study reveals that confinement modulates the ability of cells to sense and respond to haptotactic cues and provides a framework to understand how cells navigate complex and dynamic environments.

POSTER 51 presented by:

NAME: Margherita Gallano

GROUP: Cellular and molecular mechanobiology

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Nuclear Envelope Remodeling and Mechanosensing Mechanisms under Stretch

Margherita Gallano^[1,2], Aina Albajar Sigalés^[1,2], Evelyn Coderch Bifet^[1], Miguel González Martín^[1,2], Nimesh Chahare^[1,3], Anabel-Lise Le Roux^[1], Pere Roca-Cusachs^[1,2]

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Mechanical forces drive cell function in multiple scenarios, often via the plasma membrane (PM). Under mechanical stimulation, the PM exhibits a wide range of responses including changes in membrane tension, lipid packing, and order, and the rearrangement of membrane folds of different types. These membrane responses affect lipid associated proteins, triggering downstream signaling processes that could also occur in other cellular membranes. The case of the nuclear envelope (NE) is particularly interesting as mechanical forces reach the cell nucleus. The NE can indeed respond to force, as shown by the activation of NE-binding enzyme cPLA2 in response to NE tension. However, the phenomenology of NE responses to force, remain essentially unknown. In this project, we aim to characterize the dynamic response of the NE under stretch or compression and unravel related mechanosensing and downstream signaling.

POSTER 52 presented by:

NAME: Laura Faure

GROUP: Cellular and molecular mechanobiology

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

3D micropatterned traction force microscopy: a technique to control three-dimensional cell shape while measuring cell-substrate force transmission

*Laura M. Faure, Manuel Gómez-González, Ona Baguer, Jordi Cornelles, Elena Martínez, Marino Arroyo, Xavier Trepas, Pere Roca-Cusachs
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Cell shape and function are intimately linked, in a way that is mediated by the forces exerted between cells and their environment. The relationship between cell shape and forces has been extensively studied for cells seeded on flat 2-dimensional (2D) substrates, but not for cells in more physiological three-dimensional (3D) settings. Here, we demonstrate a technique called 3D micropatterned traction force microscopy (3D- μ TFM) to confine cells in three-dimensional wells of defined shape, while simultaneously measuring the forces transmitted between cells and their microenvironment. This technique is based on the 3D micropatterning of polyacrylamide wells and on the calculation of 3D traction force from their deformation. With 3D- μ TFM, we show that MCF10A breast epithelial cells exert defined, reproducible patterns of forces on their microenvironment, which can be both contractile and extensile. We further show that cells switch from a global contractile to extensile behaviour as their volume is reduced. Our technique enables the quantitative study of cell mechanobiology with full access to 3D cellular forces while having accurate control over cell morphology and the mechanical conditions of the microenvironment.

POSTER 53 presented by:

NAME: Jorge Oliver-De La Cruz

GROUP: Cellular and molecular mechanobiology

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Substrate Stiffness Regulates Tau Nuclear Localization In Neurons

*Jorge Oliver-De La Cruz¹, Yara Hinojosa Grajales¹, Mar Altadill Cordero¹, Pere Roca-Cusachs^{1,2}*¹ *Institute for Bioengineering of Catalonia, The Barcelona Institute for Science and Technology, Barcelona, Spain.*² *Facultat de Medicina, Universitat de Barcelona, Barcelona, Spain.*

Despite extensive research, the fundamental causes of neuronal loss in neurodegenerative diseases, such as Alzheimer's disease (AD), remain unidentified. Notably, while many of these diseases are associated with alterations in the brain's mechanical properties, the effects of these changes on adult neurons are poorly characterized.

Therefore, to investigate neuronal mechanoresponse, we developed a novel protocol to differentiate SH-SY5Y cells into neuron-like cells by combining biochemical induction with genetic control of the master gene NGN2 expression. This approach allowed for the rapid differentiation of cells into a more mature phenotype, as confirmed by immunostaining and PCR analysis. These differentiated neuronal-like cells were cultured on polyacrylamide gels of varying rigidities, functionalized with laminin and fibronectin. The neurons displayed morphological adaptations, including reduced spreading and shorter neurite length on compliant substrates. These changes were associated with decreased polarity in the distribution of stable microtubules, indicated by a lower neurite/soma ratio. Importantly, we discovered that soft environments induced the nuclear accumulation of Tau phosphorylated at residues 202 and 205, a phenomenon observed in AD patients. Using specific cytoskeletal inhibitors, we demonstrated that this nuclear accumulation of the phosphorylated Tau occurs downstream of microtubule stability but not actin stability.

These findings were further validated using a human induced pluripotent stem cell (hiPSC) line derived from an AD patient with the APOE4/E4 genotype and its isogenic line corrected to APOE3/E3. Neurons with the APOE4/E4 genotype exhibited alterations similar to those observed in AD patients, including endosome accumulation, increased Tau phosphorylation in the perisoma and nucleus, and reduced polarity in the distribution of stable tubulin. These mislocalizations were exacerbated when cultured on substrates with lower rigidities for both genotypes.

Our findings reveal a potential role of neuronal mechanotransductive pathways in the development of pathological events associated with neurodegenerative diseases, opening the possibility for new therapeutic strategies targeting these pathways.

POSTER 54 presented by:

NAME: Özge Özgüç

GROUP: Integrative cell and tissue dynamics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

A bottom-up model to study biomechanics of human amniotic sac developmentÖzgüç, Özge¹, Wilson, Thomas¹, Treppe, Xavier^{1,2,3}¹ Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute for Science and Technology (BIST), Barcelona, Spain.² Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Barcelona, Spain.³ Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

Despite its scientific and clinical significance, the dynamics of human embryo development remain largely mysterious, especially after implantation because post-implantation human embryos are mostly inaccessible to experimentation, and they are precious biological materials that are too limited to build robust quantitative studies. Recent studies have shown the ability of human pluripotent stem cells (hPSCs) to self-organize and establish a lumen, resulting in the formation of a cyst-like structure with many hallmarks of the human epiblast in culture. These synthetic models provide a unique opportunity to study instructive mechanical cues and the biomechanics of early human development in a simple and highly quantitative platform. In this project, we tackle the lumenogenesis of epiblasts using hPSCs with engineering perspective on synthetic morphogenesis. Studying the dynamics of this event can unravel the mechanisms underlying the formation of the amnion, a signaling center that guides embryonic development from peri-implantation to gastrulation. To achieve this, we are developing a microfluidic human amniotic sac model system with which we can measure and control the mechanics of the amniotic cavity during formation, development, and homeostasis, in addition to studying mechanotransduction in further epiblast differentiation.

POSTER 55 presented by:

NAME: Alice Perucca

GROUP: Integrative cell and tissue dynamics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Understanding the mechanobiology of immune infiltration in colon cancer.

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Immune cell migration is essential for immune response to be successful. The dysregulated environment of growing cancers negatively impacts immune cell migration through the tumor microenvironment. Some of the factors that influence immune cell migration in cancer context have been extensively described. However, there is a lack of studies correlating the spatial organization of the fibroblast and the substrate tension with the migration modes of immune cells through them. Moreover, previous data in our lab showed that fibroblasts accumulated around cancer islets are under tension, and that immune cells preferentially migrate along those fibroblasts instead of infiltrating the tumor. We speculate that the high tension between aligned fibroblasts contributes to immune cells sequester in the stroma. Our approach to answer these questions consists in observing T-cell migration through geometrically organized liver fibroblasts, using specific fibroblasts forming nematic monolayers. Nematic defects are known to have conserved force patterns. Thus, by controlling the spatial organization of the fibroblasts we will standardize their alignment and force fields while investigating the T-cell migration.

POSTER 56 presented by:

NAME: Marc Rico-Pasto

GROUP: Associated researcher

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Circulation-on-a-Chip: Cell Survival Under Pro-Apoptotic Mechanical Cues in Metastasis*M. Rico-Pasto^{1,2}, V. Batto^{1,2}, J. Ibáñez¹, P. Fernandez-Nogueira¹, J. Alcaraz^{1,2,3}*¹ *Unit of Biophysics and Bioengineering, Department of Biomedicine, School of Medicine and Health Sciences, Universitat de Barcelona, Barcelona 08036, Spain*² *Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute for Science and Technology (BIST), Barcelona 08028, Spain*³ *Thoracic Oncology Unit, Hospital Clinic Barcelona, Barcelona 08036, Spain*

Lung adenocarcinoma (ADC) is a major contributor to brain metastasis, which is a major cause of cancer death. Conventional treatments and studies have focused on primary and secondary tumors, leaving the circulating tumor cells (CTCs) understudied. Likewise, how CTCs overcome the pro-apoptotic cues posed by the hydrodynamic conditions within the circulation (shear stress, hydrostatic pressure) in their journey to the brain remains largely unknown. To address this gap of knowledge, we designed a microfluidic based circulation-on-a-chip model that reproduces key physiological hydrodynamic features of the middle cerebral artery to investigate how disseminated cancer cells, survive within this hostile mechanical environment. Using this system, we examined the survival of different lung cancer cell lines exhibiting low (H441) or high (H460) metastatic potential. Cell lines with traits of monocytes (THP-1) and T-cells (Jurkat) were used as positive controls. Our results demonstrate that the aggressive H460 cell line survives significantly more than non-aggressive H441 cells, yet all cancer cells consistently exhibited lower survival than both THP-1 and Jurkat cells at all times examined. In all conditions, cell viability as a function of time could be modeled with a simple biophysical model based on an exponential decay and an activated process corresponding to the energetic barrier that cells need to overcome to activate death response during the hydrodynamics posed by the circulation. These results provide a proof-of-principle of a novel circulation-on-a-chip model, which provides a suitable tool to study CTCs by identifying the mechanisms underlying the enhanced survival of cancer cells with high metastatic potential, by screening drugs against their aberrant survival as well as to generate models of residual disease by enriching a population of cells with enhanced survival within the circulation.

POSTER 57 presented by:

NAME: Janet van der Graaf Mas

GROUP: Integrative cell and tissue dynamics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Experimental model of the mechanobiology of the immunocompetent tumor ecosystem*van der Graaf Mas, Janet¹, Perucca, Alice¹, Dalaka, Eleni¹, Guillamat, Pau¹, Calon, Alexandre², Trepac, Xavier¹*¹ Integrative Cell and Tissue Dynamics, IBEC² Cancer Research Program, Hospital del Mar Medical Research Institute (IMIM), 08003 Barcelona, Spain.

The progression of a tumor and its response to therapy depend on the evolution of a complex tumor microenvironment (TME) that includes cancer cells, cancer-associated fibroblasts (CAFs), endothelial cells and immune cells, among others. In many types of solid tumors, the TME prevents immune cells from eliminating the disease by inhibiting their capacity to migrate into the tumor, which typically leads to immunotherapy failure. Growing evidence shows that the main contributors to immune exclusion are CAFs, who envelop the tumor and exert active compression on cancer cells. This physical barrier, together with the secreted signaling proteins and ECM components create a barrier to immune infiltration. To understand how mechanochemical interactions drive tumor progression and how they determine treatment efficacy, there is a need to develop experimental models of the tumor ecosystem. Here we present the proof-of-concept of our technology, which we call Tumor Ecosystem On Chip (TEOC), a microphysiological system that enables control and measurement of the mechanobiology of the TME, focusing on immune exclusion. By using surface micropatterning, we are able to control cell alignment and investigate the effects of CAFs architecture and mechanobiology on immune cell migration. This will allow us to investigate by which mechanisms the spatial alignment of CAFs and the mechanobiological properties of the TME determine immune infiltration.

POSTER 58 presented by:

NAME: Thomas Wilson

GROUP: Integrative cell and tissue dynamics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Unveiling the 3D Mechanics of Tubular Epithelial Structures for Biohybrid Devices

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All surfaces of our body, both internal and external, are covered by thin cellular layers called epithelia. Epithelia are responsible for fundamental physiological functions such as morphogenesis, compartmentalization, filtration, transport, environmental sensing, and protection against pathogens. These functions are determined by the three-dimensional (3D) shape and mechanics of epithelia. One commonly formed shape are 3D tubular structures, such as blood vessels, lung bronchioles, and kidney renal tubules. However, the mechanisms behind how epithelial tubes behave under differing flows and geometric conditions remains poorly understood. We aim to address this question by developing a technology to engineer the elementary building blocks of epithelial morphogenesis and to reverse-engineer their mechanics. With a combination of micropatterning, sacrificial matrices, and microfluidics, we will implement a new experimental platform to sculpt epithelial tubes of a controlled geometry. We apply these engineering principles to build biohybrid devices based on 3D epithelia and create a microfluidic channel composed of epithelial tissue that can be imaged with high spatial-temporal resolution. Through this approach, we will map the stress and strain tensors and luminal pressure, and then to control these variables from the subcellular to the tissue levels. We aim to perform full experimental study of the 3D mechanics of tubular epithelial channels, and to unveil the mechanical principles and underlying forces by which these tissues adopt and sustain their shape. Our study establishes a new approach for engineering epithelial biohybrid microfluidic devices.

POSTER 59 presented by:

NAME: Shuqin Chen

GROUP: Smart nano-bio-devices

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Convective Dynamics of Swarming Enzymatic Nanomotors

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Enzymatic nanomotors harvest kinetic energy through the catalysis of chemical fuels. When a drop containing nanomotors is placed in a fuel-rich environment, they assemble into ordered groups and exhibit intriguing swarming behaviour akin to the self-organization observed in bacterial colonies, bioconvection of aerobic microorganismal suspensions, and the coordinated movements of fish, ants, and birds. This swarming behaviour presents numerous advantages compared to individual nanomotors, including expanded coverage and prolonged propulsion duration. However, the physical mechanisms underlying the swarming have yet to be fully elucidated. Our study investigates the formation of enzymatic swarms using experimental analysis and computational modeling. We show that the directional movement of enzymatic nanomotor swarms is due to their solutal buoyancy. We investigate various factors that impact the movement of nanomotor swarms, such as particle concentration, fuel concentration, fuel viscosity, and vertical confinement. We examine the effects of these factors on swarm self-organization to gain a deeper understanding. In addition, the urease catalysis reaction produces ammonia and carbon dioxide, accelerating the directional movement of active swarms in urea compared with passive ones in the same conditions. The numerical analysis agrees with the experimental findings. Our findings are crucial for the potential biomedical applications of enzymatic nanomotor swarms, ranging from enhanced diffusion in bio-fluids and targeted delivery to cancer therapy.

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POSTER 60 presented by:

NAME: Núria Blanco-Cabra

GROUP: Bacterial infections: antimicrobial therapies

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Novel Fluidic System With Controlled Shear Stress For Personalized Diagnostic In Biofilm-Related Infections

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Biofilms are complex microbial communities that exhibit enhanced resistance to antibiotics, posing significant challenges in treating chronic infections and necessitating personalized diagnostics for each case. The *in vitro* biofilm device (IVD) is a novel fluidic system designed to mimic dynamic conditions and control shear stress, better simulating *in vivo* environments. This device offers a straightforward and reproducible method to cultivate biofilms and assess treatment efficacy, enabling personalized strategies for managing biofilm-associated infections.

POSTER 61 presented by:

NAME: Luisa Camerin

GROUP: Nanoprobes and nanoswitches

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Photoswitchable Carbamazepine Analogs for Non-Invasive Neuroinhibition *In Vivo*

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A problem of systemic pharmacotherapy is off-target activity, which causes adverse effects. Outstanding examples include neuroinhibitory medications like antiseizure drugs, which are used against epilepsy and neuropathic pain but cause systemic side effects. There is a need for drugs that inhibit nerve signals locally and on-demand without affecting other regions of the body. Photopharmacology aims to address this problem with light-activated drugs and localized illumination in the target organ. Here, we have developed photoswitchable derivatives of the widely prescribed antiseizure drug carbamazepine. For that purpose, we expanded our method of ortho azologization of tricyclic drugs to meta/para and to N-bridged diazocine. Our results validate the concept of ortho cryptoazologs (uniquely exemplified by Carbazopine-1) and bring to light Carbadiazocine (8), which can be photoswitched between 400-590 nm light (using halogen lamps and violet LEDs) and shows good drug-likeness and predicted safety. Both compounds display photoswitchable activity *in vitro* and in translucent zebrafish larvae. Carbadiazocine (8) also offers *in vivo* analgesic efficacy (mechanical and thermal stimulus) in a rat model of neuropathic pain and a simple and compelling treatment demonstration with non-invasive illumination.

POSTER 62 presented by:

NAME: Marta Badia

GROUP: Protein phase transitions in health and disease

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

A comprehensive landscape of IAPP amyloid aggregation*Marta Badia, Benedetta Bolognesi**Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST),
Barcelona, Spain.*

Amyloids formed by the islet amyloid polypeptide hormone (IAPP) have been shown to cause pancreatic beta-cell damage, leading to a decline in insulin secretion and Type-II diabetes (T2D).

Even minor changes in the IAPP primary sequence can influence its aggregation rate. IAPP sequence in bears, which only differs in four amino acids from human IAPP, does not form amyloids. Conversely, specific single amino acid changes are enough to accelerate the IAPP aggregation rate.

Deciphering how mutations modify IAPP aggregation can help us gain a mechanistic understanding of the process of amyloid formation of this peptide and preventively identify mutations that could increase the risk of developing T2D.

Here, we measured the impact of 1668 IAPP mutations on its ability to nucleate amyloids thanks to a multiplexed cellular assay.

Our dataset includes distinct types of mutations (substitutions, insertions, and deletions) and identifies a continuous stretch of residues (15-32) which likely builds the core of IAPP amyloids. This stretch matches the core of the first protofilaments that appear in the *in vitro* aggregation reaction. Inside this core, we find that mutations have a more drastic effect in the 22-27 NFGAIL segment, which is located in the interface between the protofilaments in IAPP fibrils.

Additionally, this mutational atlas also identifies many mutations in the N-terminal region of the peptide that increase amyloid formation, suggesting that local structural elements in this region can normally protect against aggregation.

POSTER 63 presented by:

NAME: Júlia Alcàcer Almansa

GROUP: Bacterial infections: antimicrobial therapies

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Exploring host-pathogen interactions: Unraveling the dynamics of *Pseudomonas aeruginosa* and *Burkholderia cenocepacia* coinfection in *Galleria mellonella*Júlia Alcàcer-Almansa^{1,2}, Joana Admella^{1,2}, Núria Blanco-Cabra^{1,2}, Eduard Torrents^{1,2,*}¹ Bacterial infections and antimicrobial therapies group, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Baldri Reixac 15-21, 08028 Barcelona, Spain.² Microbiology Section, Department of Genetics, Microbiology and Statistics, Faculty of Biology, Universitat de Barcelona, 643 Diagonal Ave., 08028, Barcelona, Spain.

Pseudomonas aeruginosa and *Burkholderia cenocepacia* are two multidrug-resistant opportunistic pathogens often isolated from the lungs of cystic fibrosis patients and other bronchiectasis. It is known that the presence of more than one species in an infection promotes the appearance of a network of interactions that can lead to an increase in their antimicrobial tolerance or to the evasion of the host immune system. *Galleria mellonella* has been used as an animal model throughout this study, as its primary immune response is comparable to that of mammals. In order to comprehend bacterial and host behaviors post-infection with single and dual-species bacterial cultures, the survival rate of *Galleria mellonella* after *P. aeruginosa* and *B. cenocepacia* was monitored. Additionally, the efficacy of antibiotic treatment against these infections was assessed. Also, to characterize the infection evolution, tissue-specific infection dissemination and hemocyte phagocytosis were evaluated through confocal microscopy. Finally, the infection evolution was analyzed from a molecular point of view, comparing immunity-related *Galleria mellonella* gene expression as well as bacterial virulence-related gene expression in single and dual-species infected groups. The comparison provided insights into how the presence of multiple bacterial species affects the host immune response and bacterial virulence. This comprehensive study sheds light on the intricate interplays between host and pathogens and the underlying mechanisms of polymicrobial infection, taking us a step further in the development of more effective therapeutic strategies against polymicrobial infections.

The group is supported by grants PID2021-125801OB-I00, PLEC2022-009356 and PDC2022-133577-I00 funded by MCIN/AEI/10.13039/501100011033/FEDER UE, and the CERCA programme and AGAUR-Generalitat de Catalunya (2021SGR-01545)-European Regional Development Fund (FEDER) and the Catalan Cystic Fibrosis association. J.A.-A. is thankful to MCIN for its financial support through a PRE2021-098703 grant funded by MCIN/AEI/ 10.13039/501100011033 and by "ESF Investing in your future".

POSTER 64 presented by:

NAME: Cátia D. F. Lopes

GROUP: Molecular bionics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Precision nanomedicine-enabled CRISPR-powered gene therapy for efficient amyloid- β clearance across the blood-brain barrier

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder that primarily affects the brain, leading to cognitive decline and memory impairment. Its exact cause is not fully understood, but AD is characterised by the abnormal extracellular accumulation of amyloid- β protein aggregates and intracellular tau tangles, which disrupt normal brain functioning. AD has no cure, and the available treatments have limited effectiveness in halting or reversing the progression of the disease. In addition, with the growing global ageing of the population, AD incidence and prevalence are expected to double in Europe by 2050. Therefore, AD remains a significant global health challenge, awaiting the development of a reliable, safe, and effective therapeutic option to halt disease progression and prevent cognitive failure.

Aiming to improve AD pathophysiology, our team is developing an innovative solution to modulate the process of amyloid- β clearance through the blood-brain barrier (BBB). We are developing a pioneering gene therapy that integrates CRISPR/Cas9 technology to modulate the expression of a key intervenient in amyloid- β clearance at the BBB, thus enabling the transport of amyloid- β across it. Besides leveraging the precision of CRISPR/Cas9, this innovative strategy also incorporates super-selective nanocarriers tailored to target BBB and mediate the targeted therapeutic gene delivery. By synergistically combining advanced genetic and nanotechnology strategies, our approach is anticipated to influence AD progression positively.

Here, we will present our latest *in vitro* findings. Our results show a successful modulation of target gene expression levels in brain endothelial cells – a critical step in our intervention – and a consequent significant improvement in amyloid- β transcytosis through the brain endothelium.

CRISPR Cas9 technology's precision, coupled with our nanocarriers' specificity, can potentially impact the progression of AD by influencing the process of amyloid- β clearance at the BBB. These findings contribute to our understanding of AD pathophysiology and mark a significant stride towards developing a reliable, safe, and effective therapeutic option.

POSTER 65 presented by:

NAME: Antonino Nicolò Fallica

GROUP: Nanomalaria

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Development of YAT2150 analogues as potent multistage antiparasmodial agents

Antonino Nicolò Fallica^{1,2}, Inés Bouzón-Arnáiz^{1,2}, Pau Reolid³, Elsa M. Arce³, Ana Mallo-Abreu^{3,4}, Lucía Román-Álamo^{1,2}, Claudia Camarero-Hoyos^{1,2}, Daniela Currea-Ayala^{1,2}, Marc Orozco-Querí^{1,2}, Maria Ribera², Miriam Ramírez², Yunuen Avalos-Padilla^{1,2}, Diego Muñoz-Torrero^{3,4}, Xavier Fernández-Busquets^{1,2,5}

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Malaria ranks among the most lethal parasitic diseases worldwide, with treatment efficacy declining due to increasing drug resistance against current antimalarial therapies. Addressing this issue requires identifying new compounds that disrupt multiple pathways critical to the parasite's infectiousness and survival. YAT2150, a fluorescent dye traditionally used to detect protein aggregation *in vitro*, has shown potential in this regard. Our research team discovered that YAT2150 effectively impairs protein aggregation in *Plasmodium*, thereby exerting an antiparasmodial effect ^[1]. Encouraged by these findings, we initiated a lead optimization campaign to develop more potent and less toxic derivatives of YAT2150. We identified three promising derivatives that have low toxicity for human umbilical vein endothelial cells and Caco-2 cells and exhibit enhanced antiparasmodial activity against both asexual and sexual stages of *Plasmodium*. Additionally, these compounds are strongly active against *Plasmodium falciparum* strains resistant to current antimalarial drugs. Their effect in reducing protein aggregation in live *P. falciparum* cultures was assessed using the thioflavin-T assay. The compounds demonstrated favorable *in vitro* pharmacokinetic and pharmacodynamic profiles, and preliminary assays in a *Caenorhabditis elegans* model indicated for all of them a low *in vivo* toxicity. Overall, these findings highlight the potential of YAT2150 derivatives as promising candidates for the development of new antimalarial therapies.

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Funding: This work was supported by grants PID2021-128325OB-I00, PID2020-118127RB-I00, and PDC2022-133085-I00, funded by Ministerio de Ciencia e Innovación/Agencia Estatal de Investigación (MCIU/AEI/10.13039/501100011033), which included FEDER funds.

POSTER 66 presented by:

NAME: Nina Kostina

GROUP: Bioinspired interactive materials and protocellular systems

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Harnessing nature's blueprints to design interactive synthetic cells

*Nina Kostina, César Rodríguez Emmenegger
IBEC Institute for Bioengineering of Catalonia*

Nature achieves unmatched functionality by the self-assembly of (macro)molecular building blocks in a hierarchical manner. All information necessary for the function is encoded at the molecular level. Inspired by Nature, my research focuses on building synthetic cells (SynCells), tailor-made synthetic vesicles capable of recapitulating some fundamental biological properties and performing tasks. In particular, I am interested in building SynCells that interact with natural cells to direct their fate in a programmed manner and fight pathogens. To tackle this, we have designed and synthesized new families of amphiphiles —comb-polymers and Janus dendrimers— that self-assemble into cell-mimetic vesicles termed combisomes and dendrimersomes. Although these molecules do not exist in Nature, the formed vesicles closely mimic cell membranes' thickness, flexibility, and lateral 2D organization. The unparalleled matching of biophysical properties enabled the harboring of functional components of natural membranes and even fusion with living cells to “hijack” their periphery providing an almost inexhaustible palette to design the chemical and biological makeup of the synthetic cells. The final goal is that the SynCells will recognize the bacteria, engulf it and kill it inside the endosome. Such Phagocytic SynCells can serve as active scavengers that protect implants and medical devices from bacterial colonization using mechanisms that do not cause the emergence of resistance. This concept has never been explored before, and I believe that it has a very high potential to become a new strategy for combating antibiotic-resistant bacteria and will have a high impact on medicine.

POSTER 67 presented by:

NAME: Joana Admella Pedrico

GROUP: Bacterial infections: antimicrobial therapies

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Studying *Pseudomonas aeruginosa* and *Staphylococcus aureus* infection in alveolar epithelial cellsJoana Admella ^{1,2} and Eduard Torrents ^{1,2}¹ Bacterial infections and antimicrobial therapies group, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Baldori Reixac 15-21. 08028 Barcelona, Spain.² Microbiology Section, Department of Genetics, Microbiology and Statistics, Faculty of Biology, Universitat de Barcelona, 643 Diagonal Ave., 08028, Barcelona, Spain.

Respiratory diseases like chronic obstructive pulmonary disease (COPD), asthma or cystic fibrosis (CF) are characterized by impaired lung airways that lead to an increased risk of persistent infections. *Pseudomonas aeruginosa* is one of the main pathogens involved in chronic respiratory infections. These infections are usually associated with biofilm formation, making them more challenging to reproduce and treat. *P. aeruginosa* can coexist with several microorganisms, but its partnership with *Staphylococcus aureus* is widespread in CF and COPD patients.

Finding a good *in vitro* model to study long-term *P. aeruginosa* infections is extremely difficult due to the secretion of highly virulent toxins that compromise the model within a few hours. Alveolar 3D cell cultures and bovine serum albumin (BSA) were used as a media supplement, among other optimizations; cell viability was enhanced for more than 24 hours. Within this time frame, we were able to study different aspects of the infection, delve deeper into host-pathogen interactions, and explore the relationship between *P. aeruginosa* and *S. aureus*. We also aimed to underline the importance of selecting the right bacterial strain by demonstrating the completely different behaviors and results obtained between the widely used reference strain *P. aeruginosa* PAO1 and the chronic clinical isolate *P. aeruginosa* PAET1.

Acknowledgements

The authors wish to thank Dr. Maria del Mar Cendra for developing some past work in this project. The group is supported by grants PID2021-125801OB-I00, PLEC2022-009356 and PDC2022-133577-I00 funded by MCIN/AEI/10.13039/501100011033/FEDER, UE, the CERCA programme and AGAUR-Generalitat de Catalunya (2021SGR-01545) and the Catalan Cystic Fibrosis association. JA is thankful to Generalitat de Catalunya, for its financial support through the FI program (2021FI_B00118).

POSTER 68 presented by:

NAME: Claudia Camarero

GROUP: Nanomalaria

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Discovery of a novel irresistible antimalarial drug with multiple targets altering *P. falciparum* protein homeostasis.

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Current antimalarial drugs are failing to make significant progress against the disease mainly due to Plasmodium, the parasite causing the disease, rampantly evolving resistance. Therefore, new drugs targeting unexplored weaknesses in the parasite are urgently needed. The recently developed YAT2150 emerges as a highly promising weapon against malaria, as a first-in-class drug with fast acting activity being effective against all parasite stages, including strains resistant to current treatments. It displays a potent *in vitro* activity (IC50 of around 90 nM) against blood stages, gametocytes, and even liver forms, offering broad-spectrum coverage.

Moreover, YAT2150 boasts several practical advantages: its simple two-step synthesis makes it cost-effective, while its long shelf life at room temperature ensures easy storage and distribution. Its presumed mode of action is the alteration of protein aggregation, a novel mechanism that would therefore affect multiple proteins, making resistance development less likely than for current drugs, for which point mutations can lead to resistance. Moreover, the fact that YAT2150 belongs to a chemical family with no other antimalarials described, further reduces resistance risks, and *in vitro* studies have shown this compound to be irresistible after extended incubation in highly mutation-prone strains.

Current research delves deeper into the identification of the specific proteins that YAT2150 affects and in understanding how the disruption of protein aggregation kills the parasite. Transcriptomic (RNAseq) and proteomic (Cellular Thermal Shift Assay) analyses following YAT2150 treatment of *P. falciparum* cultures identified numerous transcripts and proteins whose expression or stability were significantly altered by the drug. While a single target could not be isolated, several identified proteins were involved in crucial functions like glycolysis, the parasite's primary energy source,

and the production of extracellular vesicles, which may play a role in parasite-host interactions.

These results lie in line with the presumed antiplasmodial mechanism of action based on the disruption of multiple molecular targets, interfering with the formation of protein aggregates or condensates. YAT215's unique properties position it as a potential forerunner in the fight against malaria, holding significant promise for the development of next-generation antiplasmodial drugs.

Funding: This work was supported by grants PID2021-128325OB-I00, PID2020-118127RB-I00, and PDC2022-133085-I00, funded by Ministerio de Ciencia e Innovación/Agencia Estatal de Investigación (MCIN/AEI/10.13039/501100011033, Spain), which included FEDER funds. CEX2018-000806-S.

POSTER 69 presented by:

NAME: Valentino Barbieri

GROUP: Molecular bionics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

The effect of ligand surface distribution on the phenotypic targeting of brain endothelial cells

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Our previous research has demonstrated that polymersomes functionalized with angiopep-2 (AP2) peptides can selectively be transported across the blood-brain barrier (BBB) thanks to their interaction with the low-density lipoprotein receptor-related protein 1 (LRP1).^[1] This phenomenon can be utilized for many nanomedical applications, including the delivery of therapeutic cargos to the brain^[1] as well as the clearance of misfolded proteins via the regulation of LRP1 trafficking across the BBB.^[2]

In particular, transcytosis is only stimulated in a narrow range of the ligand-receptor pair's avidity, enabling precise targeting of the brain endothelial phenotype through accurate particle design.^[3] We rationalized this trend within the phenotypic association theory, an extension of superselectivity^[4] accounting for steric repulsive interactions.

Bastings and coworkers have shown that the spatial pattern of ligands on the surface of rigid nanoplatelets influences their superselective binding, which is maximized when the average ligand spacing matches the average receptor spacing on planar surfaces.^[5]

In this work, we develop patchy polymersomes through the co-assembly and phase separation of immiscible block-copolymers. Through this design, AP2 can be localized within specific patches by conjugating it to one of the polymeric components prior to co-assembly. By administering such formulations to brain endothelial cells, we investigate whether aligning the arrangement of AP2 on spherical nanoparticles to the cell surface clustering of the LRP1 receptor can affect binding to the brain endothelium in a similar fashion to what was observed in planar models for other ligand-receptor pairs. The introduction of surface patchiness has, therefore, the potential to further enhance the transport of nanomedicines across the BBB. Moreover, it is known that surface asymmetry combined with adequate particle modifications can also be exploited to prompt active transport mechanisms in response to several endogenous^[6] or external stimuli^[7], paving the way to an extra level of spatial control of our targeting strategy.

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POSTER 70 presented by:

NAME: Marco Basile

GROUP: Molecular bionics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

On the Amyloid- β transcytosis across the blood-brain barrier

*Marco Basile^{1,2,3}, Catia Lopes^{1,2}, Gian Marco Tuveri^{1,4}, Lorena Ruiz Perez^{1,2,5}, and Giuseppe Battaglia^{1,2,3,5,6}*¹ *Molecular Bionics Group, Institute for Bioengineering of Catalunya (IBEC), The Barcelona Institute of Science and Technology (BIST) Barcelona, (Spain).*² *Biomedical Research Networking Center in Bioengineering, Biomaterials, and Nanomedicine (CIBER-BBN), Barcelona, (Spain).*³ *Biomedicine Department, University of Barcelona, Barcelona, (Spain).*⁴ *Physics Department, University of Barcelona, Barcelona, (Spain).*⁵ *Department of Chemistry, University College London, London, (United Kingdom)*⁶ *Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, (Spain).*

The blood-brain barrier (BBB), among its multiple metabolic functions, is also responsible for regulating the transport of misfolded proteins to and from the Central Nervous System (CNS). One of the key players in such a process is low-density lipoprotein receptor-related proteins, including LRP1 and LRP8. LRP1 is known to control the shuttling of several misfolded proteins, including amyloid- β (A β). Our research has revealed that the BBB controls the trafficking of large molecules as a function of their avidity towards LRP1. High-avidity molecules can be retained within the endocytic pathway, while mid-avidity molecules are transported via tubular vesicles that are stabilised by the BAR domain protein, PACSIN2. We are currently investigating the role of other LRP receptors and developing multivalent polymeric nanoparticles (NPs) that can modulate and enhance this process to expedite the removal of A β .

POSTER 71 presented by:

NAME: Barbara Borges Fernandes

GROUP: Molecular bionics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Chemotaxis of natural and synthetic vesicles*Barbara Borges Fernandes^{1,2}, Twan Smits¹, Subhadip Ghosh¹, Ian Williams¹, Lorena Ruiz Perez¹, Giuseppe Battaglia^{1,3}**¹ Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain.**² Department of Condensed Matter Physics, University of Barcelona, Barcelona, Spain.**³ Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain*

Cells and microorganisms like bacteria use chemotaxis to move in a directed manner in response to concentration gradients of nutrients and toxins. Likewise, synthetic systems could take inspiration from nature to mimic this kind of transport.

Our investigation explored the essential elements required for observing chemotaxis within a synthetic protocell model. This model comprised a liposome with an encapsulated enzyme and a transmembrane protein, specifically α -hemolysin, which assembles into a heptameric pore within the liposome membrane, facilitating the transport of substrate and products into and out of the liposome. Consequently, when a liposome containing an encapsulated enzyme is situated in an environment with the corresponding substrate, an asymmetrical distribution of products emerges along its surface, promoting active motion. Our focus extended to examining the motion of liposomes encapsulating glucose oxidase and urease, exposed to glucose and urea concentration gradients, respectively. Liposomes lacking pores showed a drift towards low substrate concentration, explained by diffusioosmophoresis. Introducing pores resulted in a diminished velocity drift towards lower concentrations, implying in positive chemotaxis component. Notably, liposomes encapsulating urease with an α -hemolysin-to-lipids ratio of 0.5 (by mass) exhibited a reversal of drift toward higher urea concentrations.

The structure of natural vesicles, like exosomes, present a similar structure of our synthetic model based on liposomes with transmembrane protein pore and encapsulated enzymes. Exosomes derived from neural stem cells exhibit specific L-asparaginase activity, allowing them to act as independent metabolic units capable of altering the metabolic microenvironment by consuming asparagine and releasing aspartate. Then, we investigated if exosomes presented a chemotactic drift according to L-asparagine gradients.

POSTER 72 presented by:

NAME: Claudia Codano

GROUP: Molecular bionics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

A fumarate-based nanomedicine for macrophages' phenotypic modulationC. Codano^{1,2}, P. Pfeifer³, J. Muñoz Franco³, L. Ruiz Pérez^{1,4}, B. Gauthier³, and G. Battaglia^{1,5}¹ Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST), C Baldri Reixac 10-12, 08028, Barcelona, Spain² Department of Cell Biology, Physiology and Immunology, Faculty of Biology, Av. Diagonal 643, 08028, Barcelona, Spain³ CABIMER, Avda. Americo Vespucio 24. Edif. CABIMER Parque Científico y Tecnológico Cartuja 41092 - Sevilla Spain⁴ Department of Applied Physics, Faculty of Physics, University of Barcelona, Barcelona, Spain⁵ Catalan Institution for Research and Advanced Studies (ICREA), Passeig de Lluís Companys, 23, 08010, Barcelona, Spain

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The inflammatory process aims to eliminate cell injury causes, clear out damaged cells and tissues, and initiate tissue repair. However, dysregulated or chronic inflammation can lead to diseases, including autoimmune disorders, chronic infections, and certain cancers. M0 macrophages ("resting") play a central role in inflammation by adopting M1 ("pro-inflammatory") or M2 ("pro-resolving") phenotypes, determining the release of specific cytokines and mediators that shape the inflammatory response. Current strategies exploit metabolic reprogramming to modulate macrophage polarization and the release of inflammatory mediators via specific pathways. Nrf2 functions as an activator of antioxidant and detoxification processes and significantly influences mitochondrial and intermediary metabolism as part of its cytoprotective role. Fumarate derivatives, such as FDA-approved dimethyl fumarate (DMF), can modulate Nrf2, aiming to reprogram macrophages from an M1 to an M2 phenotype and restore the immune response. Our goal is to suppress inflammation dysregulation by leveraging the anti-inflammatory properties of fumarate combined with selective targeting of macrophages using a supramolecular nano-drug. We combined the fumarate derivative poly(propylene fumarate) (PPF) with poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) to create PMPC-PPF copolymer. PPF is a linear biodegradable polyester that releases fumarate in the cell milieu upon hydrolysis of its ester linkages. Previous studies from our group showed that monocyte-derived macrophages selectively internalize PMPC-decorated polymersomes after intravenous injection due to their interaction with receptors SRB1, CD36, and CD81, which are highly expressed on the cell surface.

PPF was synthesized by step-growth polymerization, and PMPC was added via reversible addition-fragmentation chain-transfer (RAFT). The copolymer was analyzed by nuclear magnetic resonance (NMR) to ensure quality and

conversion grade. PMPC-PPF nanoparticles were obtained by solvent-switch and characterized by Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM) to evaluate their hydrodynamic diameter and morphology.

Preliminary studies on THP1-derived macrophages treated with PMPC-PPF nanoparticles showed a reduction in TNF- α expression, a key pro-inflammatory cytokine, along with an increase in IL-10, a cytokine primarily released by pro-resolving phenotypes. These findings were validated using primary macrophages, which also exhibited reduced pro-inflammatory cytokine expression and increased anti-inflammatory cytokine expression. The results were corroborated by measurements of released cytokines and nitric oxide concentrations. Furthermore, PMPC-PPF nanoparticles were used as a prophylactic approach in an *in vivo* model of multiple sclerosis, showing a delay in disease onset compared to the untreated group.

In summary, our preliminary findings demonstrate the encouraging potential of PMPC-PPF copolymer in reducing pro-inflammatory cytokines, suggesting a promising strategy for precise metabolic modulation of inflammation.

POSTER 73 presented by:

NAME: Mauricio Cano

GROUP: Nanoscale bioelectrical characterization

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Nanoscale dielectric imaging through deep convolutional neural networks

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The computational demand for processing electrical images in AFM is particularly high, sometimes requiring weeks or even months to complete due to the complexity of the models employed and the high volume of data to process. This creates a need for methods that can accelerate the data processing workflow, and artificial intelligence can be an adequate tool for this purpose.

Techniques such as Multilayer Perceptrons have been previously used to model and replicate the processes involved in AFM data simulations. This work proposes the use of Convolutional Neural Networks (CNNs), known for their proficiency in image processing, in combination with a point-by-point Deep Neural Network (DNN). This model allows for the consideration of point-specific electrical and topographical data, while also taking into account information from its surroundings resulting in a better contextualization of each point within the image.

The proposed neural network is trained using data derived from calculations of the image that will be predicted, using as little as 3% of the sample area while achieving an R2 value of up to 0.94 in the prediction, indicating a high level of accuracy in replicating the calculation outcomes. This reduces drastically the number of calculations required, speeding up significantly processing times.

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POSTER 74 presented by:

NAME: Dario Castellana

GROUP: Biomaterials for regenerative therapies

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Radioprotective effects of Amifostine loaded in PLGA nanocarriers on 3D oral cancer models upon X-ray irradiationDario Castellana ^{1,2,3}, Agnieszka Nikitiuk ¹, Nuno Miranda Guerra Coelho ¹, Oscar Castano ^{1,2,4}, Elizabeth Engel Lopez ^{1,2,3}¹ Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain.² Ciber en Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Madrid, Spain.³ Universitat Politècnica de Catalunya, Barcelona, Spain.⁴ University of Barcelona (UB), Barcelona, Spain

Head and Neck squamous cell carcinoma (HNSCC) is the most common type of oral cancer. Nowadays, therapies include surgical ablation and systemic therapy such as radiation or chemotherapy. These effective treatments are not cancer cell-specific and thus can affect healthy cells too, inducing both acute and chronic side effects. Radiation is a systemic treatment that induces DNA damage and can impair normal cellular function, thus triggering adverse effects that reduce treatment efficacy and impact patients' quality of life. Moreover, the wound from tumor ablation can cause infection and negative implications during wound healing.

We hypothesized that a biomaterial-based drug delivery platform with the controlled kinetic release of both Amifostine, a radioprotective compound, and bioactive nanoparticles can reduce side effects and promote tissue regeneration.

Polymeric nanocarriers made of poly(lactic-co-glycolic) acid (PLGA) have been generated via a double emulsion/solvent evaporation method to encapsulate and deliver Amifostine. The negatively charged nanoparticles obtained from the synthesis had a diameter of ~250nm and an Amifostine encapsulation efficacy as high as ~80%. To test the radioprotective effect of the Amifostine particles, we developed an *in vitro* 3D model of oral mucosa cancer based on co-culture of Cal-27 and microvascular endothelial cells embedded in collagen. The 3D tumor spheroid was incubated with Amifostine before irradiation using Xlon Intl. Smart X Ray Irradiator. Radiation protocol was a single dose with varying intensity from 0 to 10 Gy and the apoptotic effect was evaluated at different time points using Alamar Blue assay.

To promote wound healing, we proved that release of biological ions, such as Zinc and Calcium, in a specific therapeutic concentration can have angiogenic and antimicrobial effects. We have synthesized Zinc and calcium nanoparticles using a custom-made sol-dry method to promote in-situ regeneration of the tumour ablated region. To further control ions' diffusion, the nanoparticles have been loaded in biodegradable polymeric electrospun fibers to control their release and increase their stability, enhancing their in-situ availability.

In conclusion, our drug-delivery platform composed of electrospun fibers and PLGA loaded Amifostine embedded in hydrogel, can control the in-situ delivery of therapeutic compounds that improve the standard therapy's overall effects and boost tissue regeneration.

POSTER 75 presented by:

NAME: Nisha Pawar Chauhan

GROUP: Molecular bionics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Liquid Phase Transmission Electron Microscopy to understand Structure and Protein Aggregation

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Protein aggregation is one of the interesting areas of research among the scientific community, because of its direct association with a wide range of human diseases [1-3]. The aggregation of amyloid- β (A β) and tau protein in the brain causes neurodegenerative diseases such as Alzheimer's or Parkinson's disease. Additionally, protein aggregation poses challenges for large-scale biopharmaceutical manufacturing and formulations of therapeutic proteins and peptides, since the unintended formation of aggregates can induce potential adverse immune reactions, decrease drug efficiency or increase production cost [4,5]. Hence, the dynamical structural changes in the protein during the protein aggregation pathway are quite important to understand. Liquid Phase Transmission Electron Microscopy (LTEM) allows us to visualize the dynamics of soft matter in a liquid environment at the nanoscale [6-9]. In this work, we have used LTEM to investigate the structural changes in A β and tau in the presence of fluid. We observed the different dynamical changes happening at the inside of protein oligomers. In addition, we observe the presence of protein monomer on the surface of A β and tau fiber, which seems to suggest the possibility of screening secondary nucleation in the proteins under study.

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POSTER 76 presented by:

NAME: David Esporrín Ubieto

GROUP: Smart nano-bio-devices

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Tuning organic nanogels for a new generation of smart nanomotors

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Extensive efforts have been made to effectively transport drugs to precise targets in the human body, but a significant challenge is the entrapment of drugs within highly viscous media such as synovial fluid (SF). To address this, scientists have encapsulated drugs within nanoparticles (NPs) and focused on surface modifications to reduce interactions with biological matrices^[1]. Recently, self-propelled NPs, or nanomotors, have emerged, enabling faster navigation through viscous media.^[2] In a previous study, we employed a tandem of silica nanomotors to overcome the biological barrier of synovial fluid (SF).^[3] Initially, we used a nanomotor based on hyaluronidase to controllably reduce SF viscosity. Following this, a urease-based nanomotor propelled particles through the medium via enzymatic activity. Although this system proved effective, it required substantial amounts of urea and approximately a 30% reduction in SF to facilitate the motion of mesoporous-based nanomotors.

In this study, we introduce an innovative approach utilizing biocompatible organic gel-based nanomotors. The organic chassis is composed of a unique combination of p-N-Isopropylacrylamide co-polymerized with p-Itaconic acid, crosslinked with varying degrees of N,N-methylenebis(acrylamide) and bis(acryloyl)cystamine. To enable self-propulsion, the chassis has undergone enzymatic modification, leveraging its catalytic activity. This novel gel-based nanomotor not only exhibits the capability to navigate intricate matrices (e.g.: SF) autonomously (with a low amount of fuel) but also possesses controlled cargo encapsulation and release functionalities. Finally, we loaded small molecules into the materials and controlled their release upon pH and temperature through SF. Also, we have conducted cytotoxicity tests on fibroblast cells to assess the materials, revealing significant biocompatibility.

All our findings underscore the potential of nanogels as a promising organic option for developing the next generation of enzymatic nanomotors. They exhibit remarkable capabilities in navigating through viscous environments to deliver therapeutic payloads precisely to their intended targets.

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POSTER 77 presented by:

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GROUP: Molecular bionics

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Polymersomes Regulating Immune Microenvironment Reduces Inflammation and Alleviates Idiopathic Pulmonary Fibrosis (IPF) by Phenotypic Targeting

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Idiopathic Pulmonary Fibrosis (IPF) is a chronic interstitial lung disease caused by excessive collagen deposition, ultimately leading to progressive pulmonary sclerosis, causing patients to lose the ability to regulate gas exchange. There are only two FDA-approved drugs for the treatment of IPF, and neither can stop or reverse disease progression or reduce mortality.

Previous studies have suggested that macrophages are innate immune cells that can regulate injury and repair in lung fibrosis models³. However, clinical therapies that directly suppress the M1 macrophage response have not been effective, suggesting that instead of directly targeting the traditionally understood activated pro-inflammatory phenotypes, it may be more useful to multivalently target different immune cell subgroups and modulate the pulmonary immune microenvironment.

There are significant differences in the expression of surface markers on bone marrow-derived interstitial macrophages (IM), monocytes, and resident alveolar macrophages (AMs), for example, human AMs highly express mannose receptor CD206³. We propose a pH-responsive polymer, poly (2-(methacryloyloxy) ethyl phosphorylcholine)-poly (2-(diisopropylamino) ethyl methacrylate) (PMPC-PDPA) polymersomes, that can target the scavenger receptor class B member 1 (SRB1) and scavenger receptor class B member 3 (CD36)⁴. By utilizing differences in the expression of surface markers of different lung immune cell subgroups, and controlling particle design features, such as particle radius, to multivalently target a subgroup of immune cells, we can interfere with innate immune activation and improve the pulmonary immune microenvironment to treat IPF, providing a new approach for the development of anti-pulmonary fibrosis drugs.

The antifibrotic effects of PMPC-PDPA polymersomes has been proved using a bleomycin (BLM)-induced mouse model of PF. Multicolor flow cytometry was also used to reveal changes in the pulmonary immune microenvironment under the intervention of PMPC-PDPA polymersomes with the disease progression, especially in terms of the number of immune cells. However, there is little information about the spatiotemporal positioning of these cells in the whole lungs. We hypothesize that cell positioning is closely related to the cellular functions of pulmonary immune cell subgroups, and therefore, the locations of different type of cells may affect their overall pro-inflammatory or anti-inflammatory activity. Therefore, the spatial interactions between PMPC-PDPA polymersomes and potential immune cells in the lung tissue during early inflammatory and late fibrotic pathological states have been demonstrated by multicolor immune fluorescence staining and tissue clearing techniques.

POSTER 78 presented by:

NAME: Kristin Fichna

GROUP: Smart nano-bio-devices

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Urease-powered nanomotors based on mesoporous silica for chemotherapeutic bladder cancer therapy

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In recent years, enormous research efforts have been made to minimize the side effects of chemotherapeutic drugs and to increase their therapeutic efficacy by developing smart drug delivery systems based on nanoparticles. Bladder cancer, for example, is one of the most common cancer types worldwide for which current therapies prolong patient survival but still show high relapse rates and thus making it urgent to improve existing therapies and deliver the drugs to their target side more efficiently. Using the catalytic reaction of enzymes that consume bioavailable fuels to propel nanoparticles, which are called enzyme-powered nanomotors, have revolutionized the field of nanomedicine. Combining nanoparticles containing therapeutic cargo and targeting moieties with the ability to move and navigate in complex biological environments has the potential to overcome current challenges in drug delivery. Here, we present urease-powered nanomotors based on mesoporous silica nanoparticles loaded with clinically relevant chemotherapeutic drugs for the potential treatment of bladder cancer. The procedure of nanoparticle synthesis to obtain homogeneous particle size distributions and ensure proper pore opening for subsequent drug loading of the nanoparticles has been optimized. To achieve the highest possible drug loading efficiency of urease-nanomotors, different drug loading approaches have been tested. Furthermore, the influence of the loaded drugs on the properties of the urease-powered nanomotors has been studied by DLS and enzymatic activity assay. Additionally, their self-propulsion in presence of relevant concentrations of urea at the single particle level as well as in swarms was monitored by optical microscopy. The biocompatibility of nanomotors was investigated, as well as their therapeutical efficacy when loaded with the drugs *in vitro* using mouse bladder carcinoma cells. We employed metabolic activity tests and viability assay for biocompatibility evaluation, and spectral flow cytometry to evaluate the ability of nanomotors to internalize into cancer cells more efficiently due to their movement in presence of fuel. These studies demonstrated the capability of urease-nanomotors to be loaded with different clinically relevant drugs without inducing changes in their collective motion. In comparison to passive nanoparticles, nanomotors showed 2.3x fold enhanced cell internalization after only 1 h of incubation. Additionally, urease-nanomotors showed excellent biocompatibility at different concentrations tested whereas drug-loaded nanomotors showed high therapeutical efficacy *in vitro*. These results are pathing the way for using drug-loaded urease-nanomotors based on mesoporous silica nanoparticles as drug delivery system for the treatment of bladder cancer.

POSTER 79 presented by:

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Drug-loaded PLGA nanomotors as a new approach for bladder cancer therapy

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Bladder cancer is the 7th most common cancer type worldwide, with over 500,000 new cases and 200,000 deaths annually. Current bladder cancer treatments are hindered by drug sedimentation and poor retention in the bladder, leading to high recurrence rates and low long-term survival. In recent years, nanomotors (NMs) have been developed as drug delivery systems for therapeutic agents.

Nanomotors are self-propelled nanoparticles capable of converting chemical energy from their surroundings into mechanical propulsion. This motion enhances their diffusion and mixing capabilities, as well as their internalization into tumors, compared to passive particles. Given these benefits, they are an excellent tool for improving bladder cancer treatment as has been demonstrated in *in vivo* experiments, where radiolabeled nanomotors reduced bladder tumor size in mice by 90%. However, the designs used so far have limitations for clinical applications due to their inorganic chassis, such as silica. Therefore, there is a need to develop new nanobots based on organic materials, which are more biocompatible, biodegradable, and FDA-approved.

In this study, we developed a new design of nanomotors based on poly (lactic-co-glycolic acid) (PLGA) to enhance the standard treatment for bladder cancer, Mitomycin C (MMC). MMC-loaded PLGA nanoparticles were synthesized using the double emulsion method. To achieve motion, the surface of the nanoparticles was modified for urease attachment by first adding polyethyleneimine (PEI) and then using glutaraldehyde as a linker for the enzyme. The polydispersity, size, and surface charge of the nanoparticles were analyzed by Dynamic Light Scattering (DLS) after synthesis and at each stage of functionalization. Additionally, the enzyme activity of the nanomotors was measured by the pH change promoted by urea catalysis using Phenol Red reagent. Moreover, motion studies were conducted by comparing nanomotors in the presence and absence of urea. After characterizing the drug-loaded nanomotors, their therapeutic efficacy was assessed in bladder cancer cells derived from mice (MB49 line) and compared with the standard treatment (free MMC), demonstrating that the motion and drug encapsulation enhanced MMC-induced cell death. Finally, the mechanisms of action of our formulation were studied by analyzing the nanomotors' cell internalization and their effect on bladder epithelial cells, showing that they can internalize into cancer cells within just one hour affecting only bladder cancer cells.

POSTER 80 presented by:

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Enzyme-Powered Nanobots for Enhanced siRNA Delivery in Bladder Cancer Therapy

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Bladder cancer (BC) is a significant global health issue, with up to 500,000 new cases and 200,000 deaths annually. Non-muscle-invasive bladder cancer (NMBIC) accounts for 75-85% of cases and has a recurrence rate of 60-70%. Traditional treatments include intravesical therapy, chemotherapy, surgery, or Bacille Calmette-Guérin (BCG) immunotherapy, however, the efficacy is limited leading to an unmet need to develop novel therapies.

In recent years, gene therapy, particularly utilizing small interfering RNA (siRNA), has emerged as a promising approach for targeted therapy. Nonetheless, siRNA faces delivery challenges due to susceptibility to degradation and limited cell membrane penetration. Overcoming these challenges requires the development of efficient delivery systems. Despite advancements, significant obstacles persist owing to various physiological barriers that nanoparticles encounter during delivery.

Nanoparticles with autonomous motion, known as nanobots (NBs), offer a promising solution. Enzyme-powered NBs, especially those utilizing physiologically relevant fuels, have shown potential for motion under *in vivo* conditions. In addition, a recent study has highlighted that there is a significant tumor accumulation of NMs in a bladder cancer mouse model.

This study focuses on the development and characterization of urease-powered NBs for the targeted delivery of siRNA to bladder cancer cells, concretely MB49 cell line. The synthesis of NBs involved the fabrication of biocompatible and biodegradable poly(lactic-co-glycolic acid) (PLGA) nanoparticles as a scaffold for siRNA loading. A layer-by-layer approach was employed to load selected siRNAs targeting genes involved in BC tumorigenesis and immunogenicity onto the PLGA NPs. Afterwards the incorporation of urease was performed to develop the NBs.

The efficacy of urease-powered NBs was evaluated through *in vitro* studies, including motion analysis, siRNA delivery efficiency, cell viability assays, and knockdown efficiency analyses. The results demonstrated successful synthesis and characterization of urease-powered NBs with optimal physicochemical properties, efficient motion in the presence of urea, and robust siRNA delivery capabilities. Importantly, cell viability remained unaffected across tested concentrations of NBs and fuel.

In conclusion, urease-powered NBs represent a promising approach for the targeted delivery of siRNA in bladder cancer therapy. Leveraging endogenous fuel sources such as urea in the urinary tract, these NBs offer a biocompatible and efficient platform for siRNA delivery, addressing critical challenges associated with conventional therapeutic modalities.

POSTER 81 presented by:

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GROUP: Smart nano-bio-devices

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Boosting urease nanomotors efficiency: purity, stability and motion*María López-Carpio¹, Daniel Sánchez-deAlcázar¹, Samuel Sánchez^{1,2}**¹ Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST), Baldori I Reixac 10- 12, 08028 Barcelona, Spain.**² Institució Catalana de Recerca i Estudis Avançats (ICREA), Passeig Lluís Companys 23, 08010 Barcelona, Spain.*

Enzyme-based nanomotors (NMs) are tiny devices driven by chemical reactions that are capable of autonomous movement at the nanoscale. One of the most used enzymes for the NM's manufacturing is urease, an enzyme found in bacteria, fungi and plants, that catalyzes the hydrolysis of urea into ammonia and carbamate.

This reaction catalysis generates propulsion force that can be harnessed to move autonomously. These NMs hold significant potential applications in targeted drug delivery, such as in treating bladder cancer, where urine provides a sustainable propulsion source [1-5]. However, most of these NMs are fabricated using commercially available urease which is found impure. However, urease purification via size-exclusion chromatography enhanced the motility and performance in micromotors [1].

Our study replicated the purification process for nanoparticles and assessed stability and catalytic efficiency over time and after exposure to different pH. The characterization of purified urease demonstrated its sustained enzymatic activity and stability both when free and upon binding to nanoparticles. Furthermore, we obtained similar activity patterns observed in both free-enzyme and nanoparticle-bound conditions across the different pH levels and maximum activity around neutral pH. Additionally, we engineered self-propelled NMs with purified urease and determined their enzymatic activity. Subsequently, we investigated their motion of at both the single particle level and in swarm behaviour using custom-designed software.

On the other hand, we propose a freezing formula to preserve urease stability, aiming to store the nanomotors longer while maintaining their motion and catalytic properties, as the enzyme's durability currently starts to decline after a week. The proposed formulas proved crucial in preserving urease activity, underscoring the importance of these processes in maintaining enzyme stability.

These results present promising opportunities for advancing our understanding of the enzyme's behaviour at different conditions of pH and its functionality on nanoparticles, improving the catalytic system of nanomotors for enhancing their motion, and optimizing storage processes to improve their preservation.

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POSTER 82 presented by:

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INSTITUTION: BBT-UPC

Synergistic Antimicrobial Effects through Ion Implantation in Boston Keratoprosthesis

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The Boston keratoprosthesis (BKPro) is a medical device used in complex cases of corneal blindness, comprising a polymethylmethacrylate frontplate and a titanium (Ti) backplate. Despite patients undergoing a complex regimen of medications, infections remain a significant threat to implant retention, occurring in 17-21% of cases. These infections, whether fungal or bacterial, primarily affect the Ti backplate due to competition between corneal cells and pathogens for surface dominance. Simultaneously, the increasing challenge of antibiotic resistance is driving the search for new infection treatment alternatives. Metallic ions with antimicrobial properties emerge as promising options for implant surfaces.

This research explores the ion implantation of silver (Ag), copper (Cu), or a combination of both, aiming to confer intrinsic microbial defense to the Ti backplate of the BKPro. Surface characterization involved scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), and ion release assays to assess the effects of implantation and biological performance. Biological evaluation confirmed no adverse effects on corneal cells (HCKs) and demonstrated antimicrobial efficacy against *Pseudomonas aeruginosa* and *Candida albicans*. Coculture studies simulating realistic infection scenarios were also conducted. SEM showed no surface changes post-implantation, and XPS confirmed successful ion incorporation. Ion release assays indicated higher Cu release compared to Ag. Ag reduced *P. aeruginosa* adhesion by 80% and delayed growth by 6 hours, while Cu reduced *C. albicans* adhesion by 40% and similarly delayed growth. Co-implanted samples exhibited combined effects. Finally, coculture assays suggested that antimicrobial surfaces benefit HCKs in surface competition.

In conclusion, ion implantation stands as a powerful tool for customizing implant surface characteristics, thereby enabling the creation of implants with intrinsic antimicrobial defense, enhancing integration, and reducing infections. Ultimately, ion implantation represents a promising alternative to prevent infections in patients implanted with BKPro, thereby mitigating the need for additional antibiotics or drugs.

POSTER 83 presented by:

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GROUP: Molecular bionics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

***In vitro* modeling of blood-brain barrier breakdown in amyloid-beta induced inflammation**Nicola Manicardi^{1,2}, Marco Basile^{1,3}, Claudia Codano^{1,4}, Cátia D. F. Lopes¹, Giuseppe Battaglia^{1,5}¹ Molecular Bionics Group, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain.² Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy.³ Biomedicine Department, University of Barcelona, Barcelona, Spain.⁴ Department of Cell Biology, Physiology and Immunology, University of Barcelona, Barcelona, Spain.⁵ Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain.

Alzheimer's Disease (AD) is a neurodegenerative disorder characterised by accumulation of abnormal aggregates of amyloid-B (AB), tau neurofibrillary tangles, and neuroinflammation. The accumulation of these misfolded proteins induces a chronic activation of microglia – the brain's resident immune cells – resulting in a chronic brain inflammatory state that impairs the blood-brain barrier (BBB) function, thus, further contributing to AD progression.

BBB is a fundamental structure of the central nervous system (CNS) and is composed by tightly packed brain endothelial cells, supported by the direct interaction of pericytes and astrocytes. This structure highly regulates the entry of substances into the brain and the clearance of various brain metabolites, like Ab, thus contributing to the maintenance of brain homeostasis. Therefore, BBB dysfunction is usually associated with disease onset and progression, rendering BBB a key player for most brain disorders, such as AD. Despite the consensus that microglia chronic activation in AD impairs BBB function, the mechanisms involved are still unclear. Therefore, this study aims to establish an *in vitro* model of the BBB under inflammatory conditions, particularly those mimicking AD, to clarify the interplay between microglia, AB, and BBB. By understanding how the BBB breaks down in AD, we hope to identify new targets for therapeutic interventions aimed at controlling the onset and progression of AD.

To establish this *in vitro* BBB model, we are employing a transwell platform where a monolayer of mouse brain endothelial cells (bEnd.3) is cultured alone or co-cultured with mouse microglia cells (BV-2). The activation of BV-2 cells as well as the expression of key BBB receptors involved in AB metabolism were evaluated by qPCR, ELISA, and Proximity Ligand Assay (PLA), after incubation with different AB aggregation forms (e.g., monomeric, oligomeric and fibrillary). In addition, the effect of AB-activated microglia on BBB integrity was assessed by measuring transendothelial electrical resistance (TEER), dextran permeability and tight junctions expression by immunocytochemistry.

Our preliminary results show that incubation of AB aggregates changes the phenotype of microglia, which seems to be dependent on contact time. On the other side, AB aggregates do not directly impact BBB integrity but induces a decrease in the expression of key receptors for AB clearance. In addition, our PLA results indicate that these receptors are directed for intracellular degradation, potentially caused by AB binding.

Our findings show the potential of this *in vitro* model to elucidate the mechanisms through which microglia affects the integrity of the BBB in the presence of AB aggregates. Therefore, it can be an important tool to identify new markers of BBB dysfunction in the context of AD, and can be also explored as a screening platform for potential therapeutics aiming at modulating chronic inflammation and restoring BBB integrity in AD.

POSTER 84 presented by:

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GROUP: Bacterial infections: antimicrobial therapies

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Discovery of novel transcriptional regulators involved in the regulation of ribonucleotide reductases in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a highly adaptable opportunistic pathogen that exhibits both acquired and innate antibiotic resistance mechanisms. Due to its survival capability in various environments, discovering new therapeutic strategies is essential. Ribonucleotide reductases (RNRs), essential enzymes for dNTP synthesis, have become promising targets for fighting *P. aeruginosa* infections. There are three main classes of RNRs, each distinguished by how their radical is generated, the metal required, cofactor type, structure, and oxygen needs. *P. aeruginosa* harbors all three classes and understanding them is crucial for comprehending its metabolic adaptability.

It is known that class Ia (nrdAB) and class II (nrdJab) are regulated by AlgR, a positive transcriptional regulator that controls mucoidy in *P. aeruginosa*. NrdR, the master negative regulator of RNR, regulates all three classes, while Anr Dnr, a negative regulator related to anaerobiosis, is involved in the regulation of class II and class III (nrdDG). However, there are still significant gaps in our understanding of the regulatory network of the three RNR classes.

This project aims to discover new transcriptional regulators through genomic, transcriptomic, and proteomic approaches. It has been suggested that DnaA, AmrZ, and other transcriptional regulators could be involved in the regulation of these pathways. Comprehensive studies of these regulators are necessary to elucidate the complex regulatory networks and hierarchical organization of these factors. Understanding these interactions is crucial for developing effective strategies to combat *P. aeruginosa* infections.

The group is supported by grants PID2021-125801OB-I00, PLEC2022-009356 and PDC2022-133577-I00 funded by MCIN/AEI/10.13039/501100011033/FEDER, UE, the CERCA programme and AGAUR-Generalitat de Catalunya (2021SGR-01545) and the Catalan Cystic Fibrosis association. A.M-M. is thankful to Generalitat de Catalunya, for its financial support through FI (2023 FI_B 00313).

POSTER 85 presented by:

NAME: Víctor Mejías Pérez

GROUP: Molecular bionics

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Deciphering the Metabolite Code: Peptide-Guided Delivery to Antigen-Presenting CellsVictor Mejias ^{1,2,3,*}, Joana Fort ^{2,4,5}, Manuel Palacín ^{2,4,5}, Giuseppe Battaglia ^{1,6,7}, and Iris L. Batalha ^{1,**}¹ Institute for Bioengineering of Catalunya (IBEC), The Barcelona Institute of Science and Technology (BIST) Barcelona, (Spain).² Institute for Research in Biomedicine (IRB Barcelona), Barcelona Institute of Science and Technology (BIST), Barcelona (Spain).³ Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona, (Spain).⁴ Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Barcelona (Spain).⁵ Department of Biochemistry and Molecular Biomedicine, Faculty of Biology, University of Barcelona, Barcelona (Spain).⁶ Biomedical Research Networking Center in Bioengineering, Biomaterials, and Nanomedicine (CIBER-BBN), Barcelona, (Spain).⁷ Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, (Spain).

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Tuberculosis (TB) remains a substantial global health burden, with an estimated 10 million new cases and around 1.5 million deaths annually. The overuse or misuse of antibiotics has led to the evolution of drug-resistant strains of *Mycobacterium tuberculosis* (Mtb), which are increasingly harder to treat due to their resistance to multiple first and second-line drugs. Even though nanomedicine holds promise in addressing TB treatment challenges, particularly related to the targeted and sustained delivery of drugs and the effective crossing of biological barriers, the direct targeting of antitubercular drugs into infected host cells remains elusive. Mucosal-associated invariant T (MAIT) cells, a distinct subset population of T cells, are able to recognise bacterial-derived metabolites, including those produced by Mtb, through their presentation by the MHC class I-like related (MR1) protein at the surface of infected cells. MR1, a conserved protein primarily located in the endoplasmic reticulum, translocates to the cell surface upon metabolite binding and association with β_2 microglobulin (β_2m), thus serving as a sensor for intracellular infections, and a very promising target for the delivery of antitubercular drugs to infected cells.

Our project leverages phage display technology to identify peptides that can bind to specific MR1-metabolite complexes. These peptides are then used to functionalize polymersomes loaded with antibiotics, guiding them towards infected cells. We successfully expressed and purified MR1 Ectodomain (MR1_ED)- β_2m complexes, both with and without acetyl-6-formylpterin (Ac-6-FP), a metabolite that has been shown to enhance MR1 presentation on the plasma membrane of cells *in vitro*. These purified complexes have been used as a target for phage display experiments, resulting in the successful identification of a range of peptide ligands that selectively bind to the unloaded and/or Ac-6-FP-loaded MR1_ED- β_2m heterodimers. These findings, further corroborated by ELISA, represent the first report of such a metabolite-dependent targeting strategy, unlocking exciting possibilities for directing therapies towards antigen-presenting cells based on their unique metabolic fingerprint.

POSTER 86 presented by:

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On the design of precision nanomedicines with dual phenotypical targeting

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Targeted therapies rely on the precise selectivity of ligands towards their target receptors. In synthetical systems, selectivity can be expertly harnessed through the implementation of multivalent systems, such as nanoparticles, when these are thoughtfully functionalized with multiple low-affinity ligands. This approach seizes the substantial influence of combinatorial entropy on binding affinity, which leads to on-off association profiles. Herein, Janus micelles, distinguished by their unique biphasic geometry and dissimilar corona properties, emerge as a multivalent scaffold to target two different phenotypes when each domain is tailored with distinct ligands, performing in the same fashion as antibodies. In this work, we present a novel ABC amphiphilic triblock copolymer system comprising poly(ethylene glycol)-polylactide-poly(N-vinylpyrrolidone), denoted as PEG-PLA-PVP, with the ability to form Janus micelles, which are generated by solution-mediated self-assembly of the PEG and PVP hydrophilic, and PLA hydrophobic blocks. Triblock synthesis lies in two steps: first, poly(ethylene glycol)-polylactide-2-bromo-2-methylpropanoate (PEG-PLA-Br) diblock macroinitiator is synthesized by the ring-opening polymerization of DL-lactide with commercial poly(ethylene glycol) and quenched with 2-bromo-isobutiryl-bromide. Following, triblock copolymer is synthesized by the atom transfer radical polymerization (ATRP) of N-vinylpyrrolidone monomer (NVP) initiated by the PEG-PLA-Br diblock produced in the former synthetic step. The characterization of the produced diblock and triblock copolymers were carried out by nuclear magnetic resonance (NMR) and gel permeation chromatography (GPC). The morphologies adopted by PEG-PLA-Br and PEG-PLA-PVP in solution were investigated by both transmission electron microscopy (TEM) and cryogenic electron microscopy (cryo-TEM), confirming the formation of micelles for both block copolymers. TEM images showed differences in the negative stained micelles generated in the diblock and triblock copolymers systems, indicating an asymmetric distribution of both hydrophilic blocks in the self-assembling of the triblock system. Presently, we are working on a library of functionalized PEG-PLA and PVP-PLA micelles to fine-tune the optimal polymer brush density and polymer tether. Our ultimate goal is to extend this research to tertiary mixture systems, combining these two functionalized diblocks with the triblock micellar scaffold. Thus, we aspire to create a versatile micellar platform to unfold the creation of nanodrugs with similar precision and efficacy observed in the field of antibody-based therapies.

POSTER 87 presented by:

NAME: Anna Panteleva

GROUP: Nanobioengineering

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Advancing Neurodegenerative Disease Research with Enhanced Brain-on-a-Chip Technology and Integrated Biosensor Systems

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Neurodegenerative disorders (NDDs), such as Alzheimer's disease, pose a significant global health challenge. Despite extensive research efforts, Alzheimer's disease remains incurable, with the majority of NDD drug candidates failing to reach the final phases of drug discovery. One of the primary challenges in developing effective treatments is the blood-brain barrier (BBB), which plays a crucial role in regulating the exchange of substances between the bloodstream and the brain. As one of the most protective membranes of the body, the BBB impedes the entry of many toxins and drugs into the brain, and its dysfunction has been closely linked to the progression of several NDDs.

Traditional animal models have been instrumental in advancing our understanding of NDDs and drug delivery mechanisms. However, these models often fall short in fully replicating the complexity of human neural responses, underscoring the need for innovative and precise tools to study neuronal activity and develop effective therapeutic interventions. Brain-on-a-chip (BoC) technology has recently emerged as a powerful tool to study neural network behavior in a controlled environment. The incorporation of a BBB component within a BoC further enhances its potential as a robust and physiologically relevant model, allowing for a more accurate mimicry of the unique properties of BBB^{1,2}.

Our research advances this field by combining a microfluidic device and multi-electrode array (MEA) technology into the BoC platform. This approach involves the co-culture of endothelial cells, pericytes, astrocytes, and neurons, creating a comprehensive and realistic model of the BBB. Through this advanced setup, we can monitor both the permeability of the BBB and neural activity in real-time using MEA electrodes³, providing deeper insights into the interactions within the brain microenvironment. The results that we have just achieved by combining these technologies and working side-by-side with our colleagues are already promising, although the development needs to be further adjusted.

This integrated approach not only enhances the physiological relevance of the BoC models but also accelerates the development of personalized therapies and drug testing for NDDs, paving the way for more effective treatment strategies.

POSTER 88 presented by:

NAME: Carles Prado

GROUP: Smart nano-bio-devices

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Exploring the Movement of Enzymatic-PLGA Nanobots in Human Skin Models

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Nanobots have brought wide attention as the next generation of vehicles for drug delivery. Their active motion and especially collective behavior have shown an enormous advantage in terms of movement in complex media, overcoming biological barriers and enhancing tumor penetration not only *in vitro* but also in *in vivo* studies. Nevertheless, there is a general concern about the side effects associated to the composition of the most extended nanobot designs, which may hinder their clinical applications. Therefore, there is still the need to develop a simple and biodegradable enzymatic nanobot based on organic materials, which would be more appealing for industry and clinicians.

Here, we present the design and characterization of a new kind of nanobot based on (poly(lactic-co-glycolic acid) (PLGA), an FDA-approved material already used in clinics. By conforming a positive layer of polyethylenimine (PEI), glutaraldehyde chemistry was used for functionalizing their surface with urease, resulting in PLGA-PEI-Urease Nanobots. By incubating these nanobots in different biological environments we have studied for the first time the differences on the degradation profile of passive and active nanobots based on enzymatic propulsion. After one week, PLGA-PEI-Urease nanobots showed some degradation in an aqueous media, and interestingly, their degradation rate increases as the pH varies during the catalytic reaction of active nanobots. The basic pH of the products of the reaction enhances the degradation of PLGA nanoparticles and PLGA-PEI-Urease nanobots.

In order to explore the safety and potential of PLGA-based nanobots as a drug delivery platform, their biocompatibility and penetration in skin models were tested. Skin is the largest organ in the body, representing a promising target site as it offers a minimally invasive administration route, but also a significant challenge due to skin natural defensive function, conforming a formidable chemical and physical barrier. *In vitro* models of epidermis and dermis were developed using neonatal Human Epidermal Keratinocytes (HEKn) for mimicking a full epidermis, while neonatal Human Dermal Fibroblasts (HDFn) were cultured in a 3D fibrin matrix. The movement and distribution of PLGA-PEI-Urease nanobots with and without the presence of fuel on the dermal and epidermal model were explored to study how far and deep do nanobots go in skin and how do they interact with the skin cells and the different biological environments present in native skin. Regarding our studies in dermal models, we have observed how active nanobots in presence of fuel (urea) are able to expand and penetrate significantly more in a 3D fibrin matrix. Thereafter, the same experiments will be carried out in a 3D dermal model with HDFn.

POSTER 89 presented by:

NAME: Romain Pastre

GROUP: Protein phase transitions in health and disease

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Combinatorial mutagenesis to investigate gain of function pathogenic variants in amyloid beta*Mireia Seuma^{1,2}, Romain Pastre¹, Andre Faure^{1,3}, Ben Lehner^{1,3,4,5}, Benedetta Bolognesi²*¹ Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain² Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Baldori Reixac 10-12, 08028, Barcelona, Spain.³ University Pompeu Fabra (UPF), Barcelona, Spain⁴ Institució Catalana de Recerca i estudis Avançats (ICREA), Barcelona, Spain⁵ Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, UK

Amyloid beta 42 (AB42) aggregates are a hallmark of all types of Alzheimer's disease (AD) and AB42 is mutated in familial forms of AD (fAD). Specific mutations in AB42 act through gain-of-pathogenic-function and increase the ability and the speed at which the peptide aggregates into amyloid fibril structures in the brains of AD patients. We asked the question whether all AB42 variants that aggregate (gain-of-function) are pathogenic and whether they lead to pathology via the same mechanism. To answer these questions, we used Deep Mutational Scanning (DMS) to quantify how 295 mutations in AB42 affect its propensity to aggregate into pathogenic amyloids. Our approach relies on the principle that variants with similar molecular consequences (aggregation score) have similar genetic interactions with other variants in the same protein. We therefore designed our experiment in such a way that we could simultaneously probe the effect of two mutations in the peptide sequence on aggregation propensity and compare it to the effect of each of these mutations on their own, producing a score that effectively quantifies their interaction. We use these scores to classify AB42 mutations into different gain-of- function categories. and find that all known familiar Alzheimer's disease (fAD) variants fall in one same class due to similar genetic interaction profiles and likely similar aggregation mechanism which is distinct from that of other non-pathogenic classes. Interaction profiling is therefore a powerful tool to uncover disease mechanisms and we believe it will significantly improve variant classification in human disease genes while also helping to generate high-throughput data needed to improve variant effect predictors.

POSTER 90 presented by:

NAME: Marina Placci

GROUP: Nanoprobes and nanoswitches

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Glucosylceramide enrichedment affects membrane nanomechanics in lipodosisPlacci, Marina ^{1,2,3*}, Remujo, Valentín¹, Muro, Silvia ^{2,4}, Giannotti, Marina I. ^{1,5,6}¹ Nanoprobes & Nanoswitches Group, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain² Targeted Therapeutics and Nanodevices Group, IBEC, BIST, Barcelona, Spain³ Biotechnology Doctorate Program, University of Barcelona (UB), Barcelona, Spain⁴ Institution of Catalonia for Research and Advanced Studies (ICREA), Barcelona, Spain⁵ CIBER-BBN, ISCIII, Barcelona, Spain⁶ Department of Materials Science and Physical Chemistry, UB, Barcelona, Spain

Gaucher disease (GD) is a lipodosis caused by several mutations on the gene encoding acid β -glucosidase, leading to aberrant accumulation of glucosylceramide (GlcCer) in cells. Despite the genetic, biochemical, and medical levels of GD are well characterized, how GlcCer accumulation in membranes alters cell biology and biomechanics processes is poorly understood. Changes in lipid composition can affect membrane's physicochemical properties (curvature, permeability, diffusion, potential, etc) and thus alter the transport processes, and directly impact on the interactions with therapeutic agents. Therefore, a complete understanding of the physical state, and mechanical properties of the lipid bilayer is essential to define their contribution to the overall membrane. GlcCer implication on the cell membrane nanomechanical properties was studied in model supported lipid bilayers (SLBs) by atomic force microscopy (AFM) and AFM-based force spectroscopy (AFM-FS). For this, liposomes composed of different combinations of dioleoyl-phosphocholine (DOPC), sphingomyelin (SME) and cholesterol (Chol), with and without GlcCer were synthesized to simulate the excess of GlcCer in GD. Samples size, polydispersity index (PDI) and Z-potential were characterized by dynamic light scattering (DLS). Size generally increased as the lipid mixtures became more complex, varying between 104-151 nm diameter, with high homogeneity (PDI<0.2) and slightly negative Z-potential values. SLBs were generated following the Vesicle-fusion Method onto mica surfaces. Their structural, topographical and nanomechanical properties were characterized. We determined the breakthrough force (F_b), as an indicator of the lateral interactions between lipid molecules. Our results show that GlcCer fully segregates from DOPC:GlcCer SLBs into well-defined, rigid domains, reflected by an extremely high F_b, which are even released from the membrane at high concentrations. When SME is present, GlcCer integrates into SME domains, increasing their packing. However, when Chol is present, a fluidifying effect is observed when comparing DOPC:Chol with DOPC:SLBs in presence, or absence, of 10-20% GlcCer. Finally, DOPC:SME:Chol±GlcCer SLBs, still have 2 different phases where the domain packing increased with GlcCer concentration, but never reaching individual GlcCer F_b levels. In general, GlcCer leads to an increased packing and mechanical resistance of the membrane domains.

Funding: Project PID2022-140459OB-I00 funded by MCIN/AEI/10.13039/501100011033/ and by FEDER "A way of making Europe". FPI fellowship program PRE2021-098133 (M.P.) funded by the Spanish Ministry of Science and Innovation MCIN AEI/10.13039/501100011033 and "ESF Investing in your future". National Polytechnic Institute of Chemical Engineering and Technology (INP-ENSIACET), Aide à la Mobilité Internationale du Ministère de l'Enseignement Supérieur et de la Recherche (MESR) (V.R).

POSTER 91 presented by:

NAME: Giulia Maria Porro

GROUP: Molecular bionics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Characterizing the modulation of the expression level of LRP1 protein in Alzheimer's Disease

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The blood-brain barrier (BBB) is a highly selective barrier composed by specialised brain endothelial cells (BECs) lining the brain vasculature. The BBB prevents the entry of toxins and pathogens into the brain and tightly regulates the trafficking and signaling of macromolecules.. In physiological conditions the low-density lipoprotein receptor-related protein 1 (LRP1), mainly expressed by BECs, prevents the accumulation of small peptides into the brain through its clearance activity. However, the decreased expression of LRP1 has been proven to actively contribute to the development of neurodegenerative disorders such as Alzheimer disease (AD). Indeed, in the AD pathogenesis the clearance of amyloid- β ($A\beta$) plaques and tau fibrils mediated by LRP1 is significantly decreased, thus leading to their extracellular accumulation and consequent toxicity.

Recent studies demonstrated that LRP1 levels in BECs may be modulated by multivalent nature nanoparticles allowing the control of LRP1 clustering and trafficking across endothelial cells. These findings are strictly dependent on the understanding of the endosomal trafficking pathways ruling the expression of LRP1 on the cell membrane.

The aim of this project is to elucidate the expression level of LRP1 at different stages of AD and how it can be modulated by the treatment of multivalent nanoparticles. Wild-type and genetically modified mice models age-matched, showing AD neuropathological features, will be used.

Also, as inflammation in AD causes endothelial cellular stress and BBB disruption, this study will focus on the analysis of the BBB integrity in the same animal models by monitoring the vascular levels of LRP1 during the progression of the disease and other biomarkers, such as LRP8, syndapin-2, Rab5, and tight-junctions proteins (e.g., occludin, claudin-3 and claudin-5). Thus, correlating the different protein level expression in a disease state compared to a physiological state.

Such a study will be a great asset for future clinical application and fundamental for a patient-tailored therapy.

POSTER 92 presented by:

NAME: Carla París Marcet

GROUP: Smart nano-bio-devices

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Self-communication of a hemin-based thermoresponsive nanomotor with an on off bubble propulsion*Carla París-Marcet¹, David Esporrín-Ubieto^{1,*}, Daniel Sánchez-deAlcázar^{1,*}, María López-Carpio¹, Samuel Sánchez^{1,2,*}*¹ *Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST), Baldiri i Reixac 10-12, 08028 Barcelona, Spain.*² *Institució Catalana de Recerca i Estudis Avançats (ICREA), Passeig Lluís Companys 23, 08010 Barcelona, Spain.*

Over the past decades, nanomedicine has emerged as a novel platform for developing advanced therapies. By manipulating the chemical structure of nanoparticles (NPs), scientists have created intelligent vehicles capable of transporting drugs and overcoming biological barriers.^[1] Recently, a new class of NPs, known as nanomotors (NMs), has been investigated for drug delivery due to their self-propelled capabilities in viscous environments, where passive nanoparticles would typically become trapped.^[2,3]

In our work, we explore smart nanogels as a new core for enzymatic-based NMs. This core is composed of a chemical crosslinking of several polymers, including N-isopropylacrylamide (p-NIPAM) and p-Itaconic acid, crosslinked with N,N'-Methylenebisacrylamide, and the catalase prosthetic group, Hemin. The p-NIPAM polymer allows the nanogel to shrink at higher temperatures, while the Hemin crosslinker catalyzes hydrogen peroxide into H₂O and O₂, generating bubbles that self-propel the NMs by a jet-like motion mechanism. We observed that bubble formation occurs only at higher pH levels. To control the pH increase, we anchored urease on the surface of the nanomotor. The urease catalyzes the conversion of urea into ammonia and carbonate, raising the pH and thereby activating Hemin. This activation leads to self-propulsion through the dismutation of H₂O₂. To optimize the composition, we tested various concentrations of Hemin to propel the NM at lower hydrogen peroxide concentrations. Finally, we will apply the best-performing material to disrupt bacterial biofilms and eradicate the bacteria using its antimicrobial properties.

*References:**[1] Tisana Kaewruethai, Chavee Laomeephol, Yue Pan, Jittima Amie Luckanagul, Gels 2021, 7(4), 228.**[2] Noelia Ruiz-González, David Esporrín-Ubieto, Ana C Hortelao, Juan C Fraire, Anna C Bakenecker, Marta Guri-Canals, Ramón Cugat, José María Carrillo, Montserrat Garcia-Batllebbó, Patricia Laiz, Tania Patiño, Samuel Sánchez, Small, 2024, 20, 11, 2309387.**[3] Meritxell Serra-Casablanques, Valerio Di Carlo, David Esporrín-Ubieto, Carles Prado-Morales, Anna C Bakenecker, Samuel Sánchez, ACS Nano, 2024, 18, 16701–16714.*

POSTER 93 presented by:

NAME: Eduard Torrents

GROUP: Bacterial infections: antimicrobial therapies

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Antimicrobial and antibiofilm activity of human recombinant H1 histones against bacterial infections

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Histones possess significant antimicrobial potential, yet their activity against biofilms remains underexplored. Moreover, concerns regarding adverse effects limit their clinical implementation. We investigated the antibacterial efficacy of human recombinant histone H1 subtypes against *Pseudomonas aeruginosa* PAO1, both planktonic and in biofilms. After the *in vitro* tests, toxicity and efficacy were assessed in a *P. aeruginosa* PAO1 infection model using *Galleria mellonella* larvae. Histones were also evaluated in combination with ciprofloxacin and gentamicin. Our results demonstrate antimicrobial activity against of all three histones against *P. aeruginosa* PAO1, with H1.0 and H1.4 showing efficacy at lower concentrations. The bactericidal effect was associated with a mechanism of membrane disruption. *In vitro* studies using static and dynamic models showed that H1.4 had antibiofilm potential by reducing cell biomass. Neither H1.0 nor H1.4 showed toxicity in *G. mellonella* larvae, and both increased larvae survival when infected with *P. aeruginosa* PAO1. Although *in vitro* synergism was observed between ciprofloxacin and H1.0, no improvement over the antibiotic alone was noted *in vivo*. Differences in antibacterial and antibiofilm activity were attributed to sequence and structural variations among histone subtypes. Moreover, the efficacy of H1.0 and H1.4 was influenced by the presence and strength of the extracellular matrix.

These findings suggest histones hold promise for combating acute and chronic infections caused by pathogens such as *P. aeruginosa*.

This study was partially supported by grants PID2021-1258010B-I00, PLEC2022-009356 and PDC2022-133577-I00 to ET, PID2020-112783GB-C21 to A.J and PID2020-112783GB-C22 to A.R., funded by Spanish Ministry of Science and Innovation MCIN/AEI/10.13039/501100011033 and "ERDF A way of making Europe", the CERCA programme and AGAUR-Generalitat de Catalunya (2021SGR01545), the European Regional Development Fund (FEDER) and Catalan Cystic Fibrosis association. The project that gave rise to these results received the support of a fellowship from "la Caixa" Foundation (ID 100010434). The fellowship code is "LCF/BQ/DI20/11780040".

POSTER 94 presented by:

NAME: Tomás Quiroga

GROUP: Protein phase transitions in health and disease

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Deep mutational scanning of SOD1 to comprehensively map the impact of mutations on protein stability*Bolognesi, Benedetta¹, Quiroga, Tomás¹**¹ Institute for Bioengineering of Catalonia (IBEC)*

We know the effect of less than 1% of all possible mutations in human proteins. When new variants are reported they are routinely categorised as Variants of Uncertain Significance (VUS), leading to poor identification of the causes of many diseases. Deep Mutational Scanning (DMS) is a powerful tool to guide clinical variant interpretation and reveal disease mechanisms. Here we used DMS in a disease Amyotrophic Lateral Sclerosis (ALS) gene, Superoxide dismutase 1 (SOD1), to map the impact of mutations on protein stability, a known SOD1-ALS phenotype. The mutational library captures the first 53 amino acids of SOD1, and includes all possible amino acid substitutions, insertions and deletions. Our results identify known SOD1-ALS variants and find that a significant part of non-reported mutations decrease the fitness score (stability), suggesting that they are likely to be pathogenic. Substitutions, insertions and deletions differ in their propensity to enhance or impair stability, but destabilising variants are enriched in the buried residues of β strands that form the β barrel structure. This comparative analysis represents the first mutational map of SOD1, highlighting the importance of specific regions in the maintenance of the SOD1 stability.

POSTER 95 presented by:

NAME: Lucia Roman Alamo

GROUP: Nanomalaria

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Development of DNA aptamers against *Leishmania infantum* GP63 protein

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Leishmaniasis is a disease affecting millions of people around the world, produced by a parasite of the genus *Leishmania*. Currently, the most commonly used diagnostic tools for parasite detection are optical microscopy and polymerase chain reaction (PCR). However, both methods require highly trained personnel and specialized facilities. Therefore, there is an urgent need to develop alternative diagnostic tools to enhance accuracy, efficiency, and accessibility compared to existing methods. Aptamers emerge as a cost-effective and more stable alternative to antibodies, rendering them a valuable approach to diagnose tropical parasitic diseases, including leishmaniasis, particularly in endemic regions. Leishmanolysin or GP63 is the major surface protein present in *Leishmania* promastigotes and constitutes one of its main virulence factors playing a role in the adhesion of the parasite to the macrophage and the survival of amastigotes. Due to the importance of this protein, DNA aptamers were developed against a *Leishmania infantum* mature form of GP63 (LiGP63m), using the systematic evolution of ligands by exponential enrichment (SELEX) method. After 7 cycles of selection, the enriched DNA sequences successfully targeted 65% of a fixed promastigote population. Twenty individual aptamer sequences were selected, and dot blot analyses confirmed the specific recognition of recombinant LiGp63m. According to fluorescence confocal and flow cytometry analysis, the selected sequences targeted above 60% of a total population of fixed promastigotes. Subsequently, the top five aptamers were further characterized using an Aptamer Linked Immobilized Sorbent Assay (ALISA) to assess their affinity to endogenous LiGP63 in a promastigote lysate, revealing binding affinities in the μM range. Our results suggest that the chosen aptamers have a high affinity and specificity for LiGP63m and are able to detect the endogenous protein in *L. infantum* promastigotes. In conclusion, the aptamers discussed here hold promise as potential diagnostic tools for leishmaniasis and as targeting molecules for functionalizing antileishmanial drug-loaded nanoparticles.

Funding: This project was supported by Fundació La Marató de TV3 (Ref. 201811) and the grants PID2021-1283250B-I00, PID2020-118127RB-I00, and PDC2022-133085-I00, funded by Ministerio de Ciencia e Innovación/Agencia Estatal de Investigación (MCIN/AEI/10.13039/501100011033, Spain), which included FEDER funds. CEX2018-000806-S.

POSTER 96 presented by:

NAME: Alessandro Ronzoni

GROUP: Molecular bionics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Dimer Or Monomer? Trying To Unravel The Structural Characteristics Of Lrp1 Protein

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LRP1 (Low Density Lipoprotein Receptor-Related Protein) is a large transmembrane receptor protein, member of the LRP family. LRP1 helps to regulate plasma lipid levels and prevents the accumulation of cholesterol-rich particles in the vasculature. This large endocytic receptor is widely expressed in various tissues and has been found to have diverse functions. Further research into the precise mechanisms through which LRP1 works will be crucial for developing targeted therapeutic strategies.

LRP1 is an integral membrane protein of 600kDa; It undergoes a proteolytic processing in the trans-Golgi compartment, that generates two subunits with molecular masses of 85 and 515kDa, respectively. Because of this characteristic, LRP1 protein undergoes a shedding process, during which the extracellular subunit detaches from the transmembrane one.

The structure of LRP1 has not yet been solved. In literature, there are some works reporting the characterisation of fragments of the protein, but none reporting the entire three-dimensional structure. The absence of a publicly available structure poses significant disadvantages for drug discovery and efforts targeting this receptor.

The structure of the LRP2 protein, a member of the LRP family closely related to LRP1, was recently solved by Shapiro et al; LRP2 is formed by a homodimer, in which the large extracellular N-terminal domains fold on each other creating a globular structure. Although in literature LRP1 has always been shown as a monomer, molecular dynamics simulations produced in our group (unpublished data), suggest that it might fold into a homodimer in a very similar conformation to LRP2.

This project aims to characterize LRP1 from a structural point of view. To do so, pure, intact, and stable, LRP1 protein needs to be obtained.

The long and winding road that leads to protein purification started from basic Molecular Biology: we generated DNA constructs with specific characteristics to enable us to express LRP1 in HEK cells and purify it. We decided to generate a construct with a twin streptag, fused to the C-terminal domain of LRP1, as a tool for protein purification. Also, we generated another construct, with an mCherry fluorophore fused in the same position as the streptag, to be able to perform microscopy analysis.

Once we had generated the main construct, we started transfecting HEK cells, and after having verified the expression through western blot analysis and confocal microscopy, we started trying to purify the protein.

We then scaled up the process, starting to work with cells in suspension, and big volumes, in order to obtain a substantial amount of protein sample. At the current stage of the project, we are optimizing the purification process, trying to achieve good protein yield and stable conditions, aiming to perform Electron Microscopy and properly characterize the structure of the protein.

As a natural following step, the obtained structure would be compared with the computed structure generated in our group, to analyse differences and similarities.

Also, with a stable purified protein, we aim to perform phage display experiments to identify new ligands of LRP1.

POSTER 97 presented by:

NAME: Zahra Saeidikia

GROUP: Molecular bionics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Combining Liquid Phase TEM and Molecular Simulations to study Misfolded Protein Aggregation in Alzheimer's Disease

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Alzheimer's disease (AD) remains a critical global health challenge, affecting approximately 55 million people worldwide and contributing to 60-70% of dementia cases. It is now understood that brains affected by AD typically contain specific types of proteins known as misfolded proteins. The buildup of misfolded proteins, including amyloid- β (A β) peptides such as A β 40 and A β 42, is a key feature of AD, leading to the formation of neurotoxic oligomers, fibrils, and plaques. Understanding the aggregation pathways of A β is critical for developing effective diagnostic and therapeutic strategies.

Liquid Phase Transmission Electron Microscopy (LPTM) offers a groundbreaking approach to visualize dynamic processes in situ, preserving the native state of the specimens under study. This technique is particularly advantageous for studying the real-time self-assembly of misfolded proteins, such as A β and Tau, associated with AD. The investigation herein proposed utilizes LPTM to visualize the structure of hydrated proteins as well as its aggregation process providing novel findings non available with other techniques.

We combine LPTM with all-atom molecular dynamics simulations to complement the structural data with dynamic insights. This integrated approach enables us to observe the formation and evolution of A β 42 monomers, dimers, and larger oligomers over time through simulations.. Such monomers exhibit a broad distribution of conformations, which correlate well with the globular structures observed in LPTM images and videos. As the peptides aggregate, we track the formation of hydrogen bonds and changes in the radius of gyration, providing a detailed view of the oligomerization pathway.

Employing these methods opens new avenues for investigating the stages of A β aggregation and offer critical insights for therapeutic interventions in Alzheimer's disease.

POSTER 98 presented by:

NAME: Daniel Sánchez de Alcázar Melendo

GROUP: Smart nano-bio-devices

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Enhancing nanomotor stability: the role of enzymatic protection*Daniel Sánchez-deAlcázar¹, Andoni Rodríguez-Abetxuko², Ana Beloqui², María López-Carpio¹, Samuel Sánchez^{1,2}**¹ Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST), Baldori I Reixac 10-12, 08028 Barcelona, Spain.**² Institució Catalana de Recerca i Estudis Avançats (ICREA), Passeig Lluís Companys 23, 08010 Barcelona, Spain.**³ POLYMAT and Department of Applied Chemistry, University of the Basque Country (UPV/EHU), Donostia San Sebastián, Spain. IKERBASQUE, Bilbao, Spain.*

In the last few decades, advancements in nanotechnology have paved the way for harnessing the power of enzymes through their integration with micro- nanoparticles, endowing them with self-propulsion features^[1-3]. Among these enzymes, catalase (CAT) has gained significant prominence due to its intrinsic properties, i.e., high turnover number and dismutation of hydrogen peroxide into water and oxygen bubbles which drive to enhanced motion properties by means of jet-like mechanism or buoyancy effect^[4]. Recently they have been successfully applied in biomedical applications^[5]. However, enzymes stability is often compromised, leading to a loss of activity when exposed to various environment, i.e., high temperature or organic solvents. These issues significantly limit its potential application in biomedicine and environmental and pollution research. In this regard, single enzyme nanogels (SENs) is an emerging technology which provides polymeric mantle around the enzyme protecting them from the media. It has been reported that this technology could increase the enzyme stability against temperature and organic solvents in addition to potential functionalities for further applications^[6-8].

Here, we show the synthesis of catalase nanogels (CAT@NGs) functionalized with amine groups and its immobilization covalently onto mesoporous silica nanoparticles (MSNPs) to fabricate for first time CAT@NGs-based nanomotors (NMs). The preliminary results showcased not only the preservation of catalytic properties in the CAT@NGs but also upon immobilization we demonstrated its ability to exhibit enhanced self-propulsion at the single particle level and collective behavior (swarm). Finally, we have demonstrated that the motion remains unaffected by temperature and organic solvents, attributed to the polymeric protection surrounding the enzyme. Moreover, the CAT@NGs-based NMs preserved their self-propulsion capability over long periods, demonstrating the high potential of SENs to stabilize and protect enzymes against aggressive media. These results suggest potential applications in environmental contexts, as NMs might navigate through various media with pollutants while retaining their catalytic activity.

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POSTER 99 presented by:

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GROUP: Molecular bionics

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Incorporating Physics-informed Neural Networks into a Physiologically Based Pharmacokinetic Model for functionalized nanoparticles biodistribution prediction

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The study aims to enhance model-informed drug discovery by incorporating Physics-informed Neural Networks (PINN) into a Physiologically Based Pharmacokinetic (PBPK) mathematical Model. It represents a step toward AI-driven development of the next-generation nanomedicine with optimized targeting potential, multifunctionality, and the ability to overcome biological barriers.

There is a significant demand for quantitative tools to assess the biodistribution, potential toxicity, and efficacy of nanomedical products. Physiologically based pharmacokinetic (PBPK) modeling provides tools for describing *in vivo* absorption, distribution, metabolism, and excretion (ADME) of nanoparticles administered to a model organism. It involves describing the physics of the metabolic processes in terms of a system of ordinary differential equations (ODE).

However, PBPK models typically require sufficient assumptions and simplifications implying limits to describe nanoparticle (NP) surface chemistry, vasculature hydrodynamics, and immune system response.

Furthermore, modern deep learning architectures, such as transformers, convolutional, or recurrent neural networks, remain inapplicable for complex biological systems as they lack robustness and ability to generalize well due to in-vivo experimental dataset size limits, signal-to-noise ratio, and the high cost and complexity of data acquisition.

In contrast, PINNs amplify the information content of noisy ground truth data being constrained to satisfy the structured information encoded in differential equations. It results in better generalization abilities, and convergence to numerical solution of the ODE, while operating in noisy and low-data regimes.

We implement a pipeline using a PBPK model (Battaglia, Xie et. al) with experimentally optimized parameters. We approximate latent (hidden) solution $\rho(t)$ of the PBPK system of differential equations $\rho_t + N[\rho] = 0$ for $t \in [0, T]$ by neural network $NN(t, W)$ with weights W , and using scientific computing technique allowing NN differentiation we minimize the loss function $L = L_\rho + L_b$ to satisfy both experimental data, boundary conditions, and to be close to the ODE numerical solution. With the PINN synthetic data are acquired, which we use to run another loop of PBPK fitting to fine-tune its parameters, providing the ground

to increase the model detalization level, and more comprehensive prediction of NP biodistribution for grid-search of candidate molecules. The system is aimed to be a closed loop for model-guided in-vivo experiments, while the model is reinforced by experiment results.

The system is aimed to be a closed loop for model-guided in-vivo experiments, while the model is reinforced by experiment results.

POSTER 100 presented by:

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Nanotechnology-driven hyperthermia in bioengineered neuroblastoma models

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Neuroblastoma (NB) is a rare pediatric cancer characterized by a high relapse rate in affected children. Innovations in this field focus on two main areas: developing accurate models and creating effective treatments. On one hand, new biomimetic models are being developed to closely mimic the clinical scenario of human NB. On the other hand, emerging therapies based on nanomedicine principles, such as hyperthermia, are gaining interest.

Hyperthermia involves exposing tumors to elevated temperatures to promote cell death or sensitization, with superparamagnetic iron-oxide nanoparticles (SPIONs) enhancing this effect. Notably, there are two key hyperthermia techniques: photothermal therapy (PTT) and magnetic hyperthermia (MH). In PTT, SPIONs are activated by local irradiation with a near-infrared laser, converting optical energy into heat. In MH, the entire tissue is subjected to alternating magnetic fields, with SPIONs generating heat by converting magnetic energy. This project aimed to evaluate these two innovative hyperthermia-based nanotherapies using tissue-engineered (TE) models that simulate human NB.

First, we developed glucose-enriched iron oxide nanoparticles to enhance tumor internalization. Next, we created three-dimensional TE models that accurately replicate the structure and components of human NB. These nanoparticles were introduced into the TE-NB models, which were then subjected to hyperthermia treatments. The effects of these treatments were monitored over time (1, 2, and 5 days).

PTT-treated models demonstrated activation of the apoptotic caspase 3-7 cascade, regardless of nanoparticle presence. However, no significant differences in cell proliferation were observed between conditions, indicating that the PTT settings were not optimal for reducing tumor growth. Conversely, MH-treated models showed activation of the apoptotic pathway only when nanoparticles were present, along with a significant reduction in cell proliferation compared to the control condition, particularly two days post-treatment. These findings suggest that MH is a promising nanotherapy-based approach.

POSTER 101 presented by:

NAME: Renato Eduardo Yanac Huertas

GROUP: Molecular and cellular neurobiotechnology

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Simulation-Guided Fabrication of Photo Printed Scaffolds for Improved Cardiac Cell Alignment in Microfluidic Environments

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Cardiovascular diseases (CVD) are heart and blood vessel conditions, the leading cause of global death. Treatment includes lifestyle changes, medication, and surgery. Regenerative therapies such as stem cells or fibroblast reprogramming aim to mitigate myocardium damage. All these therapies are tested *in vitro* at the preclinical stage, so the new microfluidic approach in the construction of *in vitro* models is currently playing an important role.

Cardiac tissue formed from cardiomyocytes, which are interwoven with collagen, is highly vascularised. Its structure results in directionally dependent electrical and mechanical properties, creating an anisotropic cardiac tissue. The cardiac muscle is characterized by its organisation and its ability to propagate electrical signals transduced in the contraction of its fibres.

In this study, we aim to print a micro scaffold using Photo 3D printing that promotes tissue alignment and can be inside a microfluidic chip. We simulated, characterized and validated, several 3D honeycomb scaffold models, varying the rods, that link the panels, in terms of length, diameter and patterns on the surface. In addition, we tested two different commercial 2PP inks. The scaffolds were characterized by SEM and the inks by tensile testing. For cell alignment, immunostaining assays were performed to label alpha-actinin and then analyzed by fast Fourier transform.

The results of the SEM analysis validate our simulations by obtaining a difference of 1.32% normalizing the deflection error concerning the length of the rods. Cell culture tests on these scaffolds showed that cardiac cells prefer an ink with a lower Young's modulus, ex: 47.7 MPa, for attachment. As for alignment, we observed that a pattern on the rods could increase it, thus inducing a better topographic signal in this 3D structure.

Based on our results, we present a robust simulation model that saves us time in the scaffold design process and 3D photo-printing and helps us to achieve a 3D structure in which cells can attach, align and proliferate. This scaffold is a good starting point from which we will add hydrogel with cells to our printed scaffold inside a microfluidic chip, this way we wish to validate our alignment hypothesis by inducing a topographical signal from the surface of the rods that aligns the cells in the hydrogel. This advance would benefit cardiac research by providing a robust microphysiological model for testing regenerative treatments and drug trials.

POSTER 102 presented by:

NAME: Maria Jose Ugarte

GROUP: Biosensors for bioengineering

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Plasmonic Biosensors to evaluate complement activation in serum of patients with myasthenia gravis*Maria J. Ugarte-Orozco ¹, Javier Ramón-Azcón ¹, Eduard Gallardo Vigo ²**¹ Institute for Bioengineering of Catalonia (IBEC) - Barcelona (Spain)**² Institut de Recerca Hospital de la Santa Creu i Sant Pau. U.A.B. - Barcelona (Spain)***mugarte@ibecbarcelona.eu*

Myasthenia Gravis (MG), an autoimmune neuromuscular disorder affecting nerve-muscle communication, is characterized by antibodies (Abs) targeting neuromuscular junction (NMJ) receptors. Most patients (80-90%) possess antibodies recognizing the acetylcholine receptor (AChR-MG), these anti-AChR Abs play a crucial role in the complement activation system (e.g., sC5b-9). These Abs lead to the formation of membrane attack complex (MAC) in the NMJ, representing the most critical pathogenic mechanism resulting in AChR depletion and subsequent muscle weakness.

^{[1][2]} Complement inhibitor therapies are emerging as effective treatments for anti-AChR Abs; however, the main problem is that considerable variability in treatment response exists, and current clinical tests have difficulty differentiating primary pathogenic mechanisms in each patient. Some patients may become unresponsive to treatment or develop chronic immunosuppression or other comorbidities, further limited by the excessive costs. ^[3]

A challenge exists due to the identification of complement-related biomarkers [3], and they cannot ascertain the effect of anti-AChR Abs on the receptor. The identification of complement activation biomarkers could facilitate the treatment selection. To address this, we aimed to develop a throughput and scalable plasmonic biointerface as an accurate diagnostic tool, measuring the release of soluble complement (sC5b-9) after its activation by anti-AChR Abs. This innovative approach could help the development of a Point-of-Care (POC) focusing on complement activation, confirming the patient's eligibility for complement inhibitor therapy, such as Eculizumab. This strategy enables label-free detection of AChR autoantibody-mediated complement activation, providing a quick and accurate diagnosis of MG.

In this study, we introduce a biosensor based on a nanostructured polycarbonate substrate from Blu-ray discs with a thin gold layer, utilizing Localized Surface Plasmon Resonance (LSPR) for analysis. It employs antibody-antigen as biorecognition elements to detect sC5b-9 in serum samples. We successfully optimized the biofunctionalization of cys-Protein G, an antibody-binding protein targeting the Fc region that enables orientation to the antibody, exposing the binding sites towards the analyte to enhance

surface sensitivity. The biosensing potential was demonstrated by the detection of sC5b-9 in culture media, achieving a limit of detection (LOD) of 0.89 ng mL. The sensor exhibited higher selectivity and sensitivity compared to existing ELISA assays. Additionally, the performance of the plasmonic biosensor was assessed with different matrices such as culture media supernatant and from a cohort of sera samples from patients provided by Sant Pau Hospital.

The label-free plasmonic biosensor we developed holds great promise for future applications in POC and portable devices within the realm of precision medicine. By offering detailed insights into the specific pathogenic mechanisms underlying MG in individual patients, our biosensor contributes to more accurate diagnosis and enables the development of personalized treatment strategies. The biosensor's high sensitivity, scalability, and compatibility with clinical workflows position it as a valuable tool for advancing bioengineering and improving patient care in the field of precision medicine.

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POSTER 103 presented by:

NAME: Akhil Venugopal

GROUP: Molecular bionics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Engineering Dynamic Lipid Vesicles with Programmable Lifetime for Controlled Cargo ReleaseVenugopal, Akhil ^{1,2}; Ghosh, Subhadip ^{1,2}; Calo, Annalisa ^{1,3}; Tuveri, Gian Marco ^{1,4}; Xie, Zhendong ^{1,4}; Battaglia, Giuseppe ^{1,5,6}; Kumar, Mohit ^{1,7,8}¹ Molecular Bionics Group, Institute for Bioengineering of Catalunya (IBEC), The Barcelona Institute of Science and Technology (BIST) Barcelona (Spain).² Biomedicine Doctorate Program, Faculty of Physics, University of Barcelona, Barcelona (Spain).³ Department of Electronic and Biomedical Engineering, University of Barcelona, Barcelona (Spain).⁴ Physics Doctorate Program, Department of Condensed Matter Physics, University of Barcelona, Barcelona (Spain).⁵ Biomedical Research Networking Center in Bioengineering, Biomaterials, and Nanomedicine (CIBER-BBN), Barcelona (Spain).⁶ Catalan Institution for Research and Advanced Studies (ICREA), Barcelona (Spain).⁷ Department of Inorganic and Organic Chemistry, University of Barcelona, Barcelona (Spain).⁸ Institute de Química Teòrica i Computacional, University of Barcelona, Barcelona (Spain).

Metabolic processes are essential for the functioning of living systems, involving the cyclic synthesis (anabolism) and degradation (catabolism) of chemical and supramolecular structures driven by the consumption of chemical energy^[1]. These highly dynamic structures which form under out-of-equilibrium (OOE) conditions define the hallmark features of life such as adaptivity and spatiotemporal control^[2]. At the cellular level, metabolic processes dynamically regulate cellular functions through the formation and degradation of constituent phospholipid molecules. Creating synthetic vesicles similar to cellular phospholipid membranes has been challenging and rarely reported in the literature [3]. Here we present a bioinspired approach for the in situ synthesis of biomimetic phospholipids and their chemical fuel driven self-assembly under physiological conditions, resulting in vesicles with a programmable lifetime. The chemical design introduces an amino-ester bond to form the lipids (anabolic reaction) through imine bond formation, which spontaneously self-assembles into vesicles. Furthermore, the simultaneous presence of lipase results in the hydrolysis of the ester (catabolic reaction) leading to disassembly. Detailed analysis through various spectroscopic and microscopic studies confirmed the continuous formation and degradation of lipid vesicles over time as long as the fuel lasts. Moreover, by varying the concentration of the lipase enzyme we could tune the lifetime of the transient vesicles from minutes to hours. We also demonstrated that these dynamic vesicles can be sustained in non-equilibrium steady states (NESS) by adding an excess amount of fuel. Detailed studies showed that NESS can be temporally controlled by the amount of fuel supplied as well as the kinetics of the enzymatic reaction. Finally, we have shown that these lipid vesicles under non-equilibrium conditions can be used for drug release by encapsulating Nile Red as a model hydrophobic drug, where the release kinetics of the encapsulated cargo molecules can be dynamically regulated for potential applications in adaptive nanomedicine.

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POSTER 104 presented by:

NAME: Marco Vigo

GROUP: Targeted therapeutics and nanodevices

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

New anti-ICAM-1 antibodies for drug delivery applicationsMarco Vigo^{1,2}, Marina Placci¹, and Silvia Muro^{1,3*}¹ Institute for Bioengineering of Catalonia (IBEC), the Barcelona Institute of Science and Technology (BIST), Barcelona 08028, Spain.² Biomedicine Doctorate Program, University of Barcelona, 08007, Spain.³ Institution of Catalonia for Research and Advanced Studies (ICREA), Barcelona 08910, Spain.

Intercellular adhesion molecule-1 (ICAM-1) is a cell-surface protein expressed during inflammation and, thus, present at most disease sites. ICAM-1 targeting is actively pursued in drug delivery for cancer, immune, genetic, cardiovascular, pulmonary, and neurological maladies. In this study, we developed new antibodies (Abs) suitable for ICAM-1 targeting and ICAM-1-mediated transport of drug nanocarriers (NCs). A phage-display Ab library was used, which rendered five unique anti-ICAM-1 sequences identified through biopanning and ELISA screening, then recombinantly expressed and purified as IgG-type proteins (named Ab1 to Ab5). All Abs bound specifically to ICAM-1-expressing cells: Ab5 was the best (30-fold over IgG), followed by Ab2, Ab3, Ab1, and Ab4 (4-fold over IgG). All Abs were efficiently coated on model polymeric nanoparticles, rendering anti-ICAM-1 NCs having 230-305 nm in diameter, 0.24-0.37 PDI, < -10 mV ζ -potential, and 135-150 Ab molecules per NC. These formulations were tested on human endothelial cells treated with TNF α to mimic ICAM-1 expression in disease. After 1 h, all formulations showed specific targeting, from 2-fold (Ab3) to 55-fold (Ab5) over IgG NCs, and uptake rates between 85 % (Ab1 NCs) and 60 % (Ab3 NCs) of all cell-interacting NCs. After 3 h, intracellular anti-ICAM-1 NCs trafficked to lysosomes, ranging between 35 % (Ab3 NCs) and 60 % (Ab1 NCs) of all cell-associated NCs. The two best overall performers, Ab2 and Ab5, were further assessed regarding transcytosis across transwell models of the brain endothelium, after verifying barrier function through the expression of cell-cell junction markers and lack of dextran leakage. Ab5 NCs was retained best by the endothelium (7 x 10⁸ vs. 3 x 10⁸ NCs/well), while Ab2 NCs exhibited superior transcytosis by 24 h (4 x 10⁹ vs. 2.5 x 10⁹ NCs/well). Therefore, this new battery of anti-ICAM-1 Abs hold relevance for ICAM-1 detection, as well as targeting and transport of drug NCs into and across ICAM-1 expressing cells, with has utility in research, diagnostics, and therapy.

POSTER 105 presented by:

NAME: Zhendong Xie

GROUP: Molecular bionics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Multiscale physiologically-based pharmacokinetics modelingZhendong Xie^{1,2,3,5}, Pan Xiang⁶, Giancarlo Franzese³, Xiaohu Tian⁶, Lorena Ruiz Perez^{1,2}, and Giuseppe Battaglia^{1,2,4,5}¹ Molecular Bionics Group, Institute for Bioengineering of Catalunya (IBEC), The Barcelona Institute of Science and Technology (BIST) Barcelona, (Spain).² Biomedical Research Networking Center in Bioengineering, Biomaterials, and Nanomedicine (CIBER-BBN), Barcelona, (Spain).³ Department of Electronics and Biomedical Engineering, Faculty of Physics, University of Barcelona (UB), Barcelona, (Spain).⁴ Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, (Spain).⁵ Huaxi MR Research Centre (HMRR), West China Hospital of Sichuan University, Chengdu, (China).

Selective drugging, popularized as the “magic bullet”, refers to the concept drugs could specifically target molecules, cells, or biotargets of interest while minimizing interactions with other parts of the body. Nanoparticles (NPs) with functionalized ligands selectively target cells bearing a certain range of receptors due to the multivalent effect. It's vital to understand NP distribution in different organs and interaction with different cell types *in vivo* to identify the most selective combination of parameters for precision drugs. We mainly investigate the distribution of poly(2-(methacryloyloxy)ethylphosphorylcholine)-poly(2-(diisopropyl-amino)ethyl methacrylate) (PMPC-PDPA) polymersome. We exploit the PMPC polymersome's promiscuous interaction with different receptors to selectively target some cell groups based on phenotypic association theory (PAT), a statistic model based on the description between nanocarriers and cell phenotype (receptor density and glycocalyx). We integrate phenotypic targeting in physiologically-based pharmacokinetics modeling (PBPK) to mimic the distribution of NPs in organs *in silico* to help us define a proper administration strategy. The PBPK is built based on the circulation system, anatomy data, and cell protein atlas to predict the distribution of the NPs among different organs considering the advection among various biological fluids, diffusion of NPs in different organs, and NPs' interaction with different cells. A non-Langmuir differential rate equation (NLDRE) is applied to extrapolate the PMPC-cell interaction kinetics based on single-cell level uptaken experiments. The association constant affinity, K_A , is derived from the PAT to reveal the selectivity of NPs to different cells. Through the experiments *in vivo*, we obtained the drug distribution among different organs, the selectivity of NPs to different cells, and some undetectable parameters, such as the glycocalyx density. Based on the parameters, we change the injected dose, the NP radius, and the polymerization of the PMPC ligand to simulate the distribution of PMPC *in silico* to help us make a better administration strategy.

POSTER 106 presented by:

NAME: Gian Marco Tuveri

GROUP: Molecular bionics

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Computational study of the Low-density lipoprotein receptor-related protein 1 (LRP1) structure and dynamics

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The brain is the most energy-expensive organ in humans, consuming around 20% of the body's metabolic resting rate. At the same time, the molecular balance that leads to biochemical reactions in the brain makes it highly delicate to alterations from the outside environment, that is, the blood circulation. Evolution led our bodies to develop a special wall between the neuronal environment and the blood flux, the so-called Blood-Brain Barrier BBB. This barrier comprises endothelial cells that tightly wrap the capillaries and apply strict control over the molecules that enter and exit the brain. In this control, the membrane proteins called receptors on the BBB play a fundamental role by binding to the molecular agents and activating the inwards/outwards transport mechanism. The present research aims to focus on the structure and function of a specific receptor, the low-density lipoprotein receptor-related protein 1, LRP1. LRP1 is composed of 4544 amino acids, around 1200 of which are involved in three long and flexible structures that contain coordinated calcium ions and are decorated with small sugar chains called glycans. These three structures are believed to have an active role in ligand binding activity and to activate a peculiar and very efficient transport mechanism. No crystal structure of LRP1 is currently available. Recently, a membrane protein closely related to LRP1, LRP2, has been resolved. The information from this crystallographic structure provides a necessary experimental insight into the super-tertiary and quaternary structures of LRP1. The LRP2 structure has been used as a template for proposing a new structure for LRP1 using a bioinformatic tool called homology modelling. The new model shows how LRP1 can assume a coiled conformation and form homodimers thanks to specific amino acids, conserved among LRP1 and LRP2, that allow the monomer-monomer interaction. Furthermore, the investigation approaches the problem using the atomistic molecular dynamics MD (Molecular Dynamics) simulations. The MD results enable us to speculate on the structural characterization of LRP1 and the time evolution of its flexible domains. The behaviour of the flexible domains in water seems to agree with the expected behaviour of the coiled conformation of LRP1 at neutral pH. Flexible domains tend to

coil, without the application of external constraints, to the same end-to-end distance observed in the dimerized structure, implying a natural tendency to converge to a particular spatial configuration. Finally, we assessed the stability of LRP1's quaternary structure with another series of MD simulations regarding the canopy portion of the proteins' complex. These simulations highlight the importance of glycans in keeping the dimer intact by creating multiple glycan-protein and glycan-glycan interactions.

POSTER 107 presented by:

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*Technology Transfer and Business Development Office at IBEC
Institute for Bioengineering of Catalonia*

IBEC performs interdisciplinary research at the highest international level to create Knowledge, Improve Health and quality of life and Generate Wealth. As a valuable piece of this engine, IBEC Technology Transfer and Business Development Office (TTBDO) performs interdisciplinary work to transfer knowledge and technologies from IBEC, helping to improve health and quality of life and generating wealth.

IBEC's TTBDO partners with IBEC researchers to develop their transferable projects, protect their inventions, and found spin-offs as tools for further project development or commercialization of certain assets. The office assists researchers building relationships with the industry at all levels, including licensing technologies and establishing R&D collaborations. IBEC's TTBDO also serves as the contact point for industries seeking to establish R&D collaborations, promoting collaborative research between public and private entities, identify researchers for service and advisory collaborations, and work on testing and refining industry-developed products. The office also facilitates special relationships, such as joint units with industry, co-establishment of spin-offs, and collaborations with venture capital companies, as well as foreign researchers in Open Lab initiatives.

TTBDO also performs back-office activities from the researcher's point of view. Activities such as attending partnering congresses, writing the IP and business sections of the grants, writing and reviewing research-, MTA-, NDA-contracts.

The collaboration with IBEC's researchers in developing transferable projects begins with scouting potentially transferrable technologies within research teams. The projects are evaluated and for those selected, a customize valorization plan is developed by the researcher, TTBDO and Project Office forming a team. The team design, run and update the valorization plan that includes the development, marketing actions and exploitation and IP strategies as well as the financial plan to ensure the transfer of the project to society. This process typically leads to successful deals for the Industrial Property, in the form of licensing agreements with established companies or with new spin-offs arising from IBEC .

IBEC's TTBDO joints effort with researchers and Research Management offices to bring IBEC solutions to society enhancing the impact of IBEC on the world.

POSTER 108 presented by:

NAME: Gender and diversity Committee

GROUP: -

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Promoting Gender Equality, Diversity, and Inclusion at IBEC: Goals and Actions of the Gender and Diversity Commission

*Gender and diversity Committee
Institute for Bioengineering of Catalonia*

At the Institute for Bioengineering of Catalonia (IBEC), we firmly believe that our institution can only reach its full potential by embracing the talents and perspectives of all individuals. Equality, diversity, and inclusion are key strengths that drive both the excellence of our research and the quality of our working environment. Our approach is based on valuing individual differences, fostering an inclusive culture where everyone feels integrated and empowered to fulfil their potential.

The IBEC Gender and Diversity Commission is committed to promoting gender equality, diversity, and inclusion at all levels, focusing on factors such as age, gender, sexual orientation, nationality, and disability, among others. Our objectives are to enhance representation, promote equal access to leadership and decision-making roles, and foster a safe, supportive environment for all employees.

To achieve these goals, we are implementing a comprehensive Equality, Diversity, and Inclusion (EDI) Plan (2024-2028) addressing key areas such as leadership, recruitment, career progression, health and work-life balance, and gender perspectives in research. IBEC EDI Plan also aims to combat discrimination and harassment, recognize intersectionality, and engage IBEC community and stakeholders in fostering a culture of change and inclusion.

Key initiatives include raising awareness and providing specific training and courses, supporting diverse talent – especially women in leadership positions – and integrating EDI practices into our policies. We also want to close the gender pay gap and ensure supervisors are equipped to support their teams effectively.

Through the implementation of these actions, we aim to create lasting cultural and structural change at IBEC, ensuring that all individuals, regardless of their background, have the opportunity to thrive and contribute to our shared success.

POSTER 109 presented by:

NAME: IBEC Sustainability Committee

GROUP: -

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

IBEC Sustainability Committee: Promoting Sustainability In Research

*IBEC Sustainability Committee
Institute for Bioengineering of Catalonia*

The Institute for Bioengineering of Catalonia (IBEC) is committed to sustainability, and so are its laboratories. In this communication we want to share some of the successful actions that we have performed with the aim of raising awareness among researchers and engaging them to make the institute more sustainable.

A list of actions proposed by the Sustainability Committee members of the institute composed by members of different profiles: Researchers, Technicians and Research Management was prioritized based on their importance and feasibility.

Most of the actions carried out at laboratory level have focused on reducing energy and water consumption, on reducing, reusing, and recycling plastic in the labs and on organizing and rationalizing the number of orders of each research group. We have also organized awareness initiatives on the framework of international days such as the World Environment Day and the Bike to Work Day. All the actions are gathered at IBEC's Sustainability website (<https://ibecbarcelona.eu/about-us/sustainable-research/>)

After being the first research institute in Spain to obtain the My Green Lab Certification seal we are now compiling the best practices of different research labs to share with the scientific community, so other research institutes and universities can implement them and become more sustainable.

If you have any sustainable suggestions or you would like to join the committee you can contact us at sustainabilitycommittee@ibecbarcelona.eu.

POSTER 110 presented by:

NAME: Core Facilities

GROUP: -

INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

MicrofabSpace and Microscopy Characterization Facilities: Empowering Research with New Technologies at IBEC

*M. Casas, M. Lantarón, M. Milozzi, E. Oriol, G. Romero, I. Oliveira and T. Galán.
Institute for Bioengineering of Catalonia*

The collaboration between research support facilities and researchers is increasingly essential. At the IBEC Core Facilities, we believe that these infrastructures should not merely act as service providers, but as key players directly involved in advancing research. The close relationship between researchers and facilities allows us to accurately identify the technological challenges that arise during their investigations and provide customized solutions to streamline the process of problem-solving.

With this collaborative approach, the MicrofabSpace and Microscopy Facilities annually undertake various pilot projects focused on overcoming everyday technical hurdles. A clear example is the acquisition and implementation of the high-resolution 3D Bioprinter QuantumX Bio by Nanoscribe (financed by MRR - Recovery, Transformation and Resilience Plan - and NextGenerationEU – Complementary Plans C.17 - funds). This equipment meets the need of fabricating structures with extremely high precision, mimicking natural biological environments and using compatible materials that cannot be processed through conventional techniques, such as lithography.

The QuantumX Bio utilizes two-photon polymerization (2PP) technology, allowing for submicrometric resolution with a minimum voxel size of 200 nm. Thanks to this capability, it is possible to print small volumes with extraordinary precision. Furthermore, since many of IBEC's research efforts focus on addressing biomedical challenges, it is crucial to employ technologies that are applicable to the biological realm. The QuantumX Bio not only stands out for its technical capabilities, but also allows printing under sterile and biological incubation conditions, enabling the presence of living organisms such as cells and bacteria. This feature makes it an ideal tool in our environment, where it is vital to study these organisms in models that faithfully simulate their natural environments before *in vivo* experimentation.

In addition, we have developed other innovative solutions:

- The fabrication of rigid epoxy polymer masters.
- The production of 3D-printed masters for hot embossing processes.
- The optimization of LED and laser lithography systems.
- Advances in confocal microscopy.
- Implementation of TIRF for particle tracking applications.



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