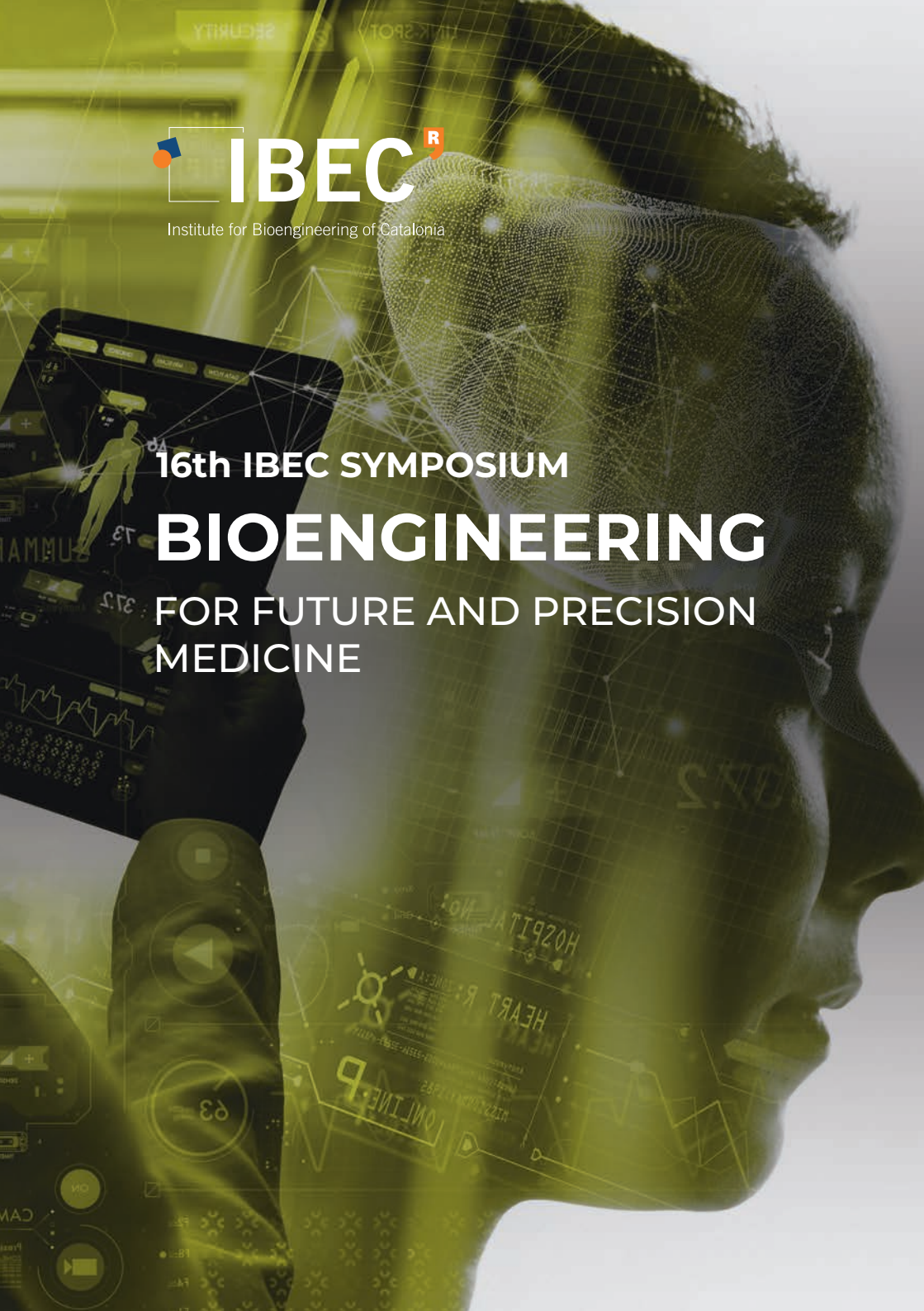
IBEC
Institute for Bioengineering of Catalonia
16th IBEC SYMPOSIUM
BIOENGINEERING
FOR FUTURE AND PRECISION MEDICINE

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BIOENGINEERING

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EXCELENCIA SEVERO OCHOA

Welcome to IBEC's 16th annual symposium

I am happy to meet you again in our annual symposium, focused on one of our three main areas of application of research at IBEC: Bioengineering for Future and Precision Medicine. I hope that you'll be stimulated and inspired by our programme of talks, posters, and networking.

Thank you very much for participating in the Symposium!

Josep Samitier

Director
Institute for Bioengineering of Catalonia (IBEC)

16th IBEC SYMPOSIUM

BIOENGINEERING

FOR FUTURE AND PRECISION MEDICINE

Programme

Tuesday 3rd October

08:00 – 09:00	Registration
09:00 – 09:15	Opening ceremony
09:15 – 09:45	*Director's presentation* IBEC's Evolution and Scientific highlights **Josep Samitier**, Institute for Bioengineering of Catalonia (IBEC)
09:45 – 10:20	Development of a first clinically viable MYC inhibitor for cancer treatment **Laura Soucek**, Vall d'Hebron Institut d'Oncologia (VHIO), Spain
10:20 – 10:50	Coffee break
10:50 – 11:25	Poster session I
11:25 – 12:00	Targeting protein aggregation in neurodegenerative diseases. **Michele Vendruscolo**, University of Cambridge. *Chair: Giuseppe Battaglia*
12:00 – 12:45	Flash presentations 1 *Chair: Zaida Álvarez*
12:45 – 13:05	Building nanobioengineered devices for translational precision diagnostics. **Lorena Dieguez**, International Iberian Nanotechnology Laboratory, INL. *Chair: Elena Martínez*

13:05 – 13:10	Group photo
13:10 – 14:00	Lunch break
14:00 – 14:35	Poster session II
14:35 – 15:10	Biofunctionality of nanomedicines to manage brain diseases overcoming barriers and drug resistance. **Bruno Sarmento**, Institute for Research and Innovation in Heatlh (i3S), University of Porto, Portugal. *Chair: Samuel Sánchez*
15:10 – 15:55	Flash presentations 2 *Chair: Santi Marco and Conrado Aparicio*
15:55 – 16:25	Round table Clinical Translation *Chair: Nuria Montserrat*
16:25 – 16:55	Poster session 3
16:55 – 17:30	Engineered viscoelasticity in stem cell microenvironments. **Manuel Salmeron**, Centre for the Cellular Microenvironment, University of Glasgow, UK. *Chair: Pere Roca-Cusachs*
17:30 – 17:40	PhD Committee Judith Fuentes, Institute for Bioengineering of Catalonia (IBEC)
17:40 – 17:50	Postdoc Committee Jorge Oliver de la Cruz, Institute for Bioengineering of Catalonia (IBEC)
17:50 – 18:00	Awards and closing ceremony


IBEC
Active Ageing

Keynote Lectures

KEYNOTE LECTURE

Development of a first clinically viable MYC inhibitor for cancer treatment

Laura Soucek

Vall d'Hebron Institut d'Oncologia (VHIO), Spain

MYC is a most wanted target in cancer therapy. However, it has long been considered an "undruggable" target, and there is no clinically approved MYC inhibitor yet. We designed and validated Omomyc, the most characterised direct MYC inhibitor to date. An Omomyc-based mini-protein therapeutic developed by Peptomyc S.L. – OMO-103 – has recently successfully completed a Phase 1 clinical study, demonstrating safety and clear signs of target engagement. Here, we present the main findings associated with biomarker discovery, both at the preclinical and clinical level, and show data on promising drug combinations to be tested in future. Indeed, the pleiotropic role of MYC in drug resistance and survival suggests that MYC inhibition could be useful to increase the efficacy of – and prevent resistance to – standard-of-care therapies.

Laura Soucek

Laura Soucek is ICREA Research Professor, co-Director of the Preclinical and Translational Research Program and Head of the Models of Cancer Therapies Laboratories at the Vall d'Hebron Institute of Oncology (VHIO) in Barcelona, Spain. In addition, she is Associate Professor at the Universitat Autonoma de Barcelona and CEO and co-founder of Peptomyc S.L. She graduated in Biological Sciences in 1996 and obtained her PhD in Genetics and Molecular Biology in 2001 at the University La Sapienza, Rome, Italy. She did her postdoc at University California San Francisco (UCSF; 2001-2006), where she was then promoted to Assistant Researcher (2006-2011). She leads her research laboratory at VHIO in Barcelona since then.

Laura is a key opinion leader in MYC biology and MYC inhibition, as well the developer of the most characterized MYC inhibitor known to date, Omomyc, which is the first MYC inhibitor to have successfully completed a Phase I clinical trial and is now ready to proceed to Phase Ib/II studies.

KEYNOTE LECTURE

Targeting Protein Aggregation in Neurodegenerative Diseases

Michele Vendruscolo

Centre for Misfolding Diseases, Yusuf Hamied Department of Chemistry, University of Cambridge

The phenomenon of protein misfolding and aggregation is associated with a wide range of human disorders, including Alzheimer's and Parkinson's diseases. A central role in these conditions is played by protein misfolded oligomers, which are among the most cytotoxic species resulting from the process of protein aggregation. It has been very challenging, however, to target these oligomers with therapeutic compounds, because of their dynamic and transient nature. To overcome this problem, I will first describe a thermodynamic-based approach based on the stabilization of the native states of proteins. I will then discuss a kinetic-based approach, which enables the discovery and systematic optimization of compounds that reduce the number of oligomers produced during an aggregation reaction. I will illustrate these strategies for the amyloid beta peptide, which is closely linked to Alzheimer's disease. As these strategies are general, they can be applied in drug discovery programs targeting any aggregating protein.

Michele Vendruscolo

Michele Vendruscolo is Professor of Biophysics, Director of the Chemistry of Health Laboratory and Co-Director of the Centre for Misfolding Diseases at the Department of Chemistry of the University of Cambridge, where he moved over 20 years ago. His work is aimed at establishing the fundamental principles of protein homeostasis and protein aggregation, and at exploiting these principles to develop methods for drug discovery in neurodegenerative diseases. He has published over 500 scientific papers and 20 patents and given over 500 invited lectures at international meetings.

KEYNOTE LECTURE

Building nanobioengineered devices for translational precision diagnostics

Lorena Diéguez

International Iberian Nanotechnology Laboratory, INL

The field of nanobioengineering has revolutionized many industries in the last decades, including healthcare, by enabling better medical imaging, more efficient therapies, vaccines and implants, and more sensitive diagnostic tools. On the other hand, microfluidics is considered a key enabling technology, allowing the precise manipulation of liquids at small scale, and the integration and automation of multiplex processes at low cost.

The Medical Devices group of INL works in close collaboration with hospitals and is dedicated to Translational Medical Research by focusing on the development of solutions based on microfluidics and nanobiosensors towards early diagnosis and better understanding of diseases.

In this talk, I will discuss the advantages of microfluidics and nanobiosensors for the efficient enrichment and multiplex characterization of disease biomarkers from body fluids.

Lorena Diéguez

Lorena Diéguez is the leader of the Medical Devices research group at INL. Her research is dedicated to Translational Medical Research in close collaboration with hospitals and focuses on the development of solutions based on microfluidics, biosensors and nanotechnology towards early diagnosis and better understanding of diseases.

She is also very interested in translating her technology from the lab to the clinic and is co-founder and CEO of the spin-off company RUBYnanomed in the field of liquid biopsy. In RUBYnanomed, her role is to bring to market a new tool for patient monitoring and therapy selection in metastatic breast cancer.

Between 2020 and 2021, she was part of the coordination team of INL's Precise Personalised HealthTech cluster. Also, since 2019 and to date, she is the Chair of the Working Group in Medical Devices at the ETPN (European Technology Platform in Nanomedicine).

She obtained her Bachelors in Physics with Major in Optoelectronics at the University of Santiago de Compostela in 2005, then completed her Masters in Nanotechnology at the University of Barcelona (UB) in 2007 and her PhD in biosensors at the UB, the Institute for Bioengineering of Catalonia and the ETH Zürich. Her postdoc at the University of South Australia (2010-2013) was devoted to the study of rare cells from biological samples using microfluidics. Lorena joined INL in 2014 as Staff Researcher, before being promoted to Research Group Leader in 2018.

She has published over 43 peer-reviewed research articles and reviews, 2 book chapters, has over 1000 citations and an h-index of 19. She has participated in 26 national and international research projects, 17 of them as PI, and 6 of them as coordinator. She has also authored 5 patents, and been invited, keynote or plenary speaker in over 30 international conferences. She has also been recipient of several international research and innovation awards.

KEYNOTE LECTURE

Biofunctionality of nanomedicines to manage brain diseases overcoming barriers and drug resistance

Bruno Sarmento

Institute for Research and Innovation in Heatlh (i3S), University of Porto

Brain diseases represent a substantial social and economic burden, currently affecting one in six individuals worldwide. Brain research has been focused of great attention in order to unravel the pathogenesis and complexity of brain diseases at the cellular, molecular and microenvironmental levels.

Due to the intrinsic nature of the brain, the presence of the highly restrictive blood-brain barrier (BBB) and the pathophysiology of most diseases, therapies can hardly be considered successful by the simple administration of drugs to a patient. Apart from improving pharmacokinetic parameters, tailoring biodistribution and reducing the number of side effects, nanomedicines are able to actively co-target the therapeutics to the brain, as well as to achieve the delivery of multiple cargos with therapeutic, diagnostic and theragnostic properties. Targeting nanomedicines can be personalized according to the disease needs, with capacity to achieve enhanced therapeutic responses across the fields of brain regeneration and neuroprotection, vascularization-related therapies, brain tumors and immunotherapies.

In the present talk, particular examples of multifunctional nanomedicines proposed by us, exhibiting capacity to cross the BBB, are described for two emergent, clinical unmet brain diseases, as glioblastoma (GBM) and multiple sclerosis.

Bruno Sarmiento

Bruno Sarmento is Principal Investigator, Group Leader and member of the Board of Directors at i3S. Bruno Sarmento is also invited Associated Professor at IUCS-CESPU. He was Visiting Professor at UniOeste (BR) between 2015-2017 and is Visiting Professor at Shanghai Jiao Tong University School of Medicine (CH).

His research is focused on developing functionalized nanomedicines, namely nanoformulations for mucosal and target permeability. He has also specialized in mucosal tissue engineering models to validate functionalized nanomedicines and to perform in vitro/in vivo correlation. He published 460 papers in international journals (total citations in Scopus 20000; H-index 71). He has supervised/co-supervised 15 Post-Docs, > 01 PhD students and > 40 MSc students. He attracted direct competitive funding worth more than 25 M€, at national and international levels. Bruno Sarmento has a strong involvement in EU projects, being WP coordinator in HORIZON-RIA 101057491-GENEGUT, 814558-2 RESTORE and ERA-Chair 951723-MOBILIsE, and coordinator of Litwin IBD Pioneers Program 937924.

Bruno Sarmento was the first Chair of the Nanomedicine and Nanoscale Delivery Focus Group of the Controlled Release Society (CRS) and is now Director-at-Large of CRS and member of CRS College of Fellows.

Javier Aparicio Calvo

Javier Aparicio Calvo es neurólogo y doctor en Medicina. Desde 2018, es el responsable de la Unidad de Epilepsia Pediátrica del Hospital Infantil Sant Joan de Déu de Barcelona. Sus principales intereses de investigación en el campo de la epilepsia son la neuroimagen en la evaluación prequirúrgica, la cirugía de la epilepsia, el diagnóstico y manejo clínico en niños y adolescentes, y las redes epileptogénicas. Es autor o coautor de más de 20 publicaciones en varias revistas de alto impacto. Ha escrito capítulos de libros sobre epilepsia y presentado más de 10 trabajos en congresos internacionales. Es miembro de la Sociedad Española de Neurología y de la Sociedad Española de Epilepsia, como también de la Red Europea de Referencia en epilepsias raras y complejas (EpiCARE).

Elena Élez

Elena Élez is the Head of the Colorectal Cancer Group at the Vall d'Hebron Institute of Oncology (VHIO) and an Associate Physician in the Medical Oncology Department of the Vall d'Hebron Barcelona University Campus (HUVH) since 2007. She is a dedicated educator, serving as a Resident Tutor since 2015 and a member of the Vall d'Hebron University Hospital's Teaching Committee since 2018, representing the autonomous community. In 2020, she earned research accreditation from the Catalan University Quality Assurance Agency. Dr. Élez is also actively involved in the +MIR section of the Spanish Society of Medical Oncology since 2018. She has contributed significantly to the development of molecular classifications for colorectal cancer based on gene expression analysis and tumor mutational profiling. Her research has been instrumental in establishing the VHIO Colorectal Cancer Group as a prominent force in colorectal cancer research under the leadership of Dr. Josep Tabernero. Her work is internationally recognized, particularly in the realm of colorectal cancer research involving the BRAFV600E mutation and microsatellite instability.

Clara Prats

Clara Prats holds a degree in Physics from the University of Barcelona and a PhD in Applied Physics and Simulation in Sciences from the Universitat Politècnica de Catalunya (UPC). She is an associate professor at the UPC, a researcher in the Computational Biology and Complex Systems group (BIOCOM-SC), president of the Interdisciplinary Health Professionals Group (Grup Interdisciplinari de Professionals vinculats amb la Salut, GIPS) and corresponding academic at the Reial Acadèmia de Medicina de Catalunya (RAMC). She has been recognized with the awards Mathematics and Society 2021 by the Fundació Ferran Sunyer i Balaguer and, together with her research team, Placa Josep Trueta al Mèrit Sanitari 2022 by the Catalan Government, Ciutat de Barcelona 2021 by the city main hall, Serrat i Bonastre 2021 and Society of Public Health of Catalonia and the Balearic Islands 2022. Her research line focuses on the use of computational modeling for the study of infectious diseases such as COVID-19 and tuberculosis, among others.

Georgina Sorrosal de Luna

Georgina Sorrosal holds a Ph.D. in Genetics and a Bachelor's degree in Biotechnology. She currently serves as the Director of the Innovation and Entrepreneurship Area at CoMB (Col·legi de Metges de Barcelona). With over 12 years of experience in healthcare innovation and science management, including leadership roles in research center spin-offs, technology transfer in hospitals, and healthcare investment funds.

KEYNOTE LECTURE

Engineered viscoelasticity in stem cell microenvironments

Manuel Salmeron-Sanchez

Centre for the Cellular Microenvironment, University of Glasgow, UK

The physical properties of the extracellular matrix (ECM) and the use of growth factors are powerful tools to control cell behaviour, including fundamental processes such as cell migration and (stem) cell differentiation. Integrins are mechanotransductors that feel and respond towards the mechanical properties of the ECM. We have developed material systems that allow simultaneous stimulation of integrins and growth factors receptors. We have engineered polymers and 3D hydrogels that unfold and assemble proteins to allow exposure of the integrin and growth factor binding regions. For example, we show the use of BMP-2 in synergy with $\alpha 5\beta 1$ integrins to promote osteogenesis and regeneration of critical-sized defects. We are interested in understanding the interplay between mechanics (viscoelasticity) of the ECM and growth factor signalling, and engineer hydrogels with independent control of elasticity and viscosity. We introduce Brillouin microscopy as a way to follow the evolution of the viscoelastic properties of cells and the engineered hydrogels in 3D in a non-invasive way and in real time.

Manuel Salmeron-Sanchez

Manuel Salmeron-Sanchez is a world leader in engineering material-based cell microenvironments for in vitro modelling, regenerative medicine and cancer (>200 papers, h-index 53). He is currently co-director of the Centre for the Cellular Microenvironment at the University of Glasgow. He leads a multidisciplinary group with 12 PhD students and 8 postdocs. Manuel was based in Valencia (Spain) until 2013 where he pioneered novel materials that triggered protein organisation (Science Advances 2016). He was awarded an ERC Consolidator Grant in 2012 to investigate materials that promote growth factor binding and their use in regenerative medicine. He moved to Glasgow in 2013 where he has assembled a multidisciplinary team that have generated internationally leading outcomes and radical new concepts: the use of viscosity to control cell behaviour (PNAS 2018); living biomaterials (bacteria-based materials) for stem cell engineering (Advanced Materials 2018); the low dose use of BMP-2 for bone regeneration (Advanced Science 2019) and the relationship between cell mechanics and metabolism in cancer (Nature Metabolism 2020). Manuel develops basic concepts that are pushed all the way to translation. He received two ERC-PoC awards used to further develop material-based bone regeneration technologies that are now being used, funded by the Sir Bobby Charlton Foundation, to help landmine survivors. Manuel has just won an ERC Advanced Grant (2022) to develop the next generation of viscoelastic materials for regenerative medicine.

Manuel has filed 3 patents (1 granted) and leads a novel clinical trial using materials for bone regeneration to be delivered in 2023. In 2017, in collaboration with vets from the small animal hospital at the University of Glasgow, he developed the technology that saved from amputation the leg of Eva –a Munsterlander run over by a car who developed an infected bone critical size defect (see https://goo.gl/1Z3r8t). Manuel is also a keen science communicator who participates in public engagement events (e.g. Science in the Café in Singapore, Spain and UK) and has recently led a team of 20 PhD students and early career researchers with an exhibit at the Science Summer Exhibition of the Royal Society (material matters – biomaterials for bone repair https://goo.gl/uG2mCg). Manuel has had his research broadcasted in national and international papers, TV (UK BBC, BBC World, Channel 4 and internationally) and Radio.

FLASH presentations

FLASH PRESENTATIONS · SESSION 1

CELL ENGINEERING

NAME	SURNAME	TITLE
Ainhoa	Ferret Miñana	3D bioengineered liver for the study of acute and chronic hepatic damage
Alba	Herrero Gómez	Non-invasive *in vivo* study on the effects of diet in mouse liver metabolism using hyperpolarization-enhanced Magnetic Resonance Spectroscopic Imaging
Lluís	Mangas Florencio	MR-compatible bioreactor for real-time metabolic analysis of 3D cell constructs for a 60 MHz benchtop NMR spectrometer
Melika	Parchehbaf Kashani	Bioprinted hydrogel-based 3D model to mimic colorectal cancer cell extravasation.

MECHANOBIOLOGY

NAME	SURNAME	TITLE
Miquel	Bosch Padrós	An optogenetic and mechanical approach to control synthetic morphogenesis
Amélie	Godeau	Mechanics Of Mouse and Human Embryo Implantation
Ignasi	Granero	Development of a sensor to study mechanotransduction in the nucleus
Jorge	Oliver-De La Cruz	Exploring neuronal mechanosensing using an optimized cellular model

FLASH PRESENTATIONS · SESSION 2

ICT FOR HEALTH

NAME	SURNAME	TITLE
Eduardo	Caballero Saldivar	Processing of Gas Chromatography Ion Mobility Spectrometry data for urine Colorectal cancer metabolomics
Eva	Martín	Fusing Clinical and Metabolomic Data for the Prediction of Ventilatory Therapies in ICU COVID Patients

NANOMEDICINE

NAME	SURNAME	TITLE
Víctor	Campo Pérez	Mycobacterium abscessus and *Pseudomonas aeruginosa* cooperate to evade immune response in the context of cystic fibrosis
Antonino Nicolò	Fallica	Targeting protein aggregation with bis(styrylpyridinium) salt derivatives: design, synthesis and preliminary biological evaluation of novel and promising antimalarial drugs
Sujey	Palma Florez	Human cortical neurons incorporation into a microfluidic model for drug screening in neurodegenerative diseases
Meritxell	Serra-Casablancas	Radionuclide therapy with accumulated urease-powered nanobots reduces bladder tumor size in an orthotopic murine model
Lena	Witzdam	Interactive coatings direct blood components to prevent clot formation in blood-contacting medical devices

FLASH PRESENTATION | CELL ENGINEERING

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

Ferret-Miñana, Ainhoa [1], De Chiara, Francesco[1] and Ramón-Azcón, Javier [1,2]
[1] Biosensors for Bioengineering Group, Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain
[2] 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 lack the ability to 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 GelMA and CMCMA, and LAP 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 recapitulate 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: Alba Herrero Gómez
GROUP: Molecular imaging for precision medicine
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Non-invasive *in vivo* study on the effects of diet in mouse liver metabolism using hyperpolarization-enhanced Magnetic Resonance Spectroscopic Imaging

Herrero Gómez, Alba [1], Gómez Cabeza, David [1], Ribas, Vicent [2], Moron, Samantha [2], Servitja, Joan-Marc [2], Azagra, Marc [1], Eills, James [1], Marco Rius, Irene [1]
[1] Institute for Bioengineering of Catalonia, Barcelona Institute of Science and Technology, Spain
[2] Institut d'Investigacions Biomèdiques August Pi i Sunyer, Clinic Barcelona, Spain

It is known for scientists and clinicians alike that the nutrient balance in one's diet is critical for the upkeep of natural and healthy metabolic processes. When there is an increase on the ingestion of both saturated and unsaturated fats, the liver starts accumulating triglycerides (TG), pathogenesis of Metabolic dysfunction-associated fatty liver disease, or MAFLD. The prevalence MAFLD is rapidly growing worldwide, as it is predicted to become the main cause of chronic liver disease ubiquitously. Pathophysiology of MAFLD indicates that one of the first aftereffects of TG accumulation in the liver is the metabolic dysregulation of vital processes such as glycolysis. This dysregulation is considered one of the hallmarks of MAFLD and a marker of early disease. However, there is no clinically available diagnostic technology that can directly monitor cell metabolism in real time non-invasively.

Hyperpolarization-enhanced magnetic resonance spectroscopic imaging (HP-MRSI) poses itself as the ideal candidate for early detection of metabolic dysfunction. Using the dissolution Dynamic Nuclear Polarization (dDNP) technique, one can track the uptake and metabolic conversion in real time of a carbon-13 labelled substrate, such as pyruvate, thanks to a signal intensity increase of over 10,000-fold compared to conventional MRSI techniques. Carbon-13 is a stable isotope, and therefore HP-MRSI is a non-invasive metabolic imaging tool that, unlike PET, does not rely on ionizing radiation. This insight enables monitoring metabolic dysregulation, detecting significant differences between study groups and proving its potential as an early detection tool for metabolic dysregulation and therefore, MAFLD. In this study, we prove HP-MRSI as a promising candidate for early-stage metabolic dysregulation detection by analyzing the metabolic differences between mice fed a standard diet and mice consuming a high fat diet (HFD) consisting of 60 kcal% fat for 20 weeks. Results show significant difference in metabolic output immediately after HP-pyruvate injection, using lactate as biomarker of inflammation and Krebs cycle dysregulation due to MAFLD.

By setting the baselines for a "healthy" and "advanced MAFLD" metabolic fingerprint, we foresee the possibility of outlining different metabolic fingerprints for different stages of the disease, allowing for precise and accurate staging for better treatment prescription as first steps toward personalized medicine.

FLASH PRESENTATION | CELL ENGINEERING

FLASH presented by:

NAME: Lluís Mangas Florencio
GROUP: Molecular imaging for precision medicine
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

MR-compatible bioreactor for real-time metabolic analysis of 3D cell constructs for a 60 MHz benchtop NMR spectrometer

Mangas-Florencio, Lluís [1,2], Herrero-Gómez, Alba [1], Azagra, Marc 1, Portela, Alejandro [2], Ellis, James [1], Marco-Rius, Irene [1,2]
[1] Molecular Imaging for Precision Medicine, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain
[2] Vitala Technologies S.L., Barcelona, Spain

In recent years, dissolution Dynamic Nuclear Polarization (dDNP) has been used to increase the sensitivity of NMR by over 10.000-fold compared to conventional NMR acquisitions. This enhancement enables tracking of metabolic conversions to study cellular metabolism in real-time and non-destructively. Bioreactors have historically been used to probe cell metabolism by DNP-NMR. Usually, bioreactors use complex set-ups and automated controlled systems, making them difficult to prepare and operate. Here we present an alternative bioreactor - a 3D printed design to simplify its in-house fabrication and assembly - for improved accessibility in research. This platform has been designed to carry out DNP-NMR analyses of 3D cell models and has been used to test a tissue engineered model of cervical cancer.

The design of the parts was done using 3D CAD software. The pieces were printed with a 3D Printer and assembled with capillary tubes to form the bioreactor. 1H-NMR acquisitions were performed on a sample of 2H2O to assess the effect of the platform on the line shape. To create the 3D cell models, 106 HeLa cells were seeded in 1% CMC cryogels. The scaffolded cells were placed into the bioreactor, cell viability and metabolism were measured. Hyperpolarized cell experiments were done injecting the substrate prepared through the 3D cell model in the bioreactor, already placed in the NMR spectrometer.

Our MR-compatible bioreactor design is ideal for easy and non-specialized fabrication, assembly, and use. The design allows for media recirculation, fast dDNP solution injection, and precise positioning of the cell-laden scaffold in the detection area of the RF coil of the spectrometer. The scaffolds used allow the cells to self-arrange and distribute inside the biomaterial while providing structure, a closer spatial distribution to that found in the native tissue. In-flow experiments enabled by this bioreactor show an 85% increase on metabolic activity when compared to those models maintained in the NMR tube in static conditions. The platform allows for detection of lactate production in the 3D cell model in DNP-NMR metabolic experiments. Moreover, the microfluidic system contributes to maintaining optimal cell culture conditions that allow for longitudinal experiments using the same sample. These results show that the platform is both simple and robust, providing a reliable tool for dDNP-NMR metabolomic studies.

FLASH presented by:

NAME: Melika Parchehbaf Kashani
GROUP: Biomimetic systems for cell engineering
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Bioprinted hydrogel-based 3D model to mimic colorectal cancer cell extravasation.

Melika Parchehbaf Kashani [1], María García-Díaz [1], Jordi Comelles [1], 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] Centro de Investigación Biomédica en Red (CIBER), Madrid, Spain
[3] Department of Electronics and Biomedical Engineering, University of Barcelona (UB), Barcelona, Spain

Colorectal cancer (CRC) metastasis is responsible for the second highest number of cancer-related deaths worldwide [1]. Metastasis initiates when tumor cells detach from the primary tumor and enter the bloodstream (intravasation). Subsequently, these cells migrate through the circulation to distant locations, where they exit the blood vessels (extravasation) and form colonies in the surrounding tissues [2, 3]. Extravasation is a critical step in cancer cell dissemination, as it facilitates the establishment of secondary metastases [4]. Vascular permeability and the pre-metastatic microenvironment in secondary tissues are crucial factors that influence the sites of extravasation and the advancement of metastasis [5, 6]. Thus, we have developed a 3D hydrogel-based model based on bioprinting that mimics the tumor microenvironment (TME) of CRC and the endothelial barrier to evaluate cancer cells extravasation.

We bioprinted the 3D hydrogel CRC model using a bioink composition based on polyethylene glycol diacrylate (PEGDA) and gelatin methacryloyl (GelMA). Human intestinal fibroblasts (HIFs) embedded in the hydrogels to mimic the stromal microenvironment, which showed impaired migration within the hydrogel bulk. To increase migration and elongation of stromal cells, we incorporated fibrinogen to obtain an interpenetrating polymer network of PEGDA/GelMA-fibrin. In this bioink, HIFs showed higher cellular viability, elongation, and migration rate.

To mimic the endothelial barrier, HUVEC cells were grown on top of the cell-embedded hydrogels in presence of VEGF, forming a monolayer with proper barrier characteristics up to 7 days as demonstrated with TEER measurements and immunostaining characterizations. The presence of HIFs enhanced the endothelial monolayer formation, whereas the presence of fibrin and VEGF promoted the endothelial sprouting and angiogenesis respectively.

To evaluate the extravasation potential of cancer cells, the bioprinted endothelial model treated with inflammatory cytokines, causing a leakier barrier with increased permeability. Then, we used SW480 cells as model circulating tumor cells and monitored their endothelial transmigration by immunostaining after 24 h incubation on top of the model. SW480 cells were able to cross the endothelial barrier and start colonizing in the hydrogel. Thus, we demonstrate that the proposed hydrogel-based model recapitulates some of the key elements of the TME in CRC and can use to study cancer cell extravasation.

FLASH PRESENTATION | MECHANOBIOLOGY

FLASH presented by:

NAME: Miquel Bosch Padrós
GROUP: Integrative cell and tissue dynamics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

An optogenetic and mechanical approach to control synthetic morphogenesis

Miquel Bosch-Padrós [xx], Kandela Ballerini [xx], Guillermo Martínez-Ara [xx], Miki Ebisuya [xx], Xavier Trepat [xx]
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A large number of processes that take place in developmental biology are currently being studied with a combined set of biological and physical tools, since morphogenesis is both controlled by physical forces and biochemical signaling. Gastrulation of embryos or organ formation are current examples of high interest in the field, involving reshaping of cell sheets and differentiation. Constriction of apical sides of cells is a known trigger of such events, but there is a knowledge gap concerning the role of cellular forces during them. By taking advantage of a novel optogenetic tool named OptoShroom3, we can spatiotemporally control apical constriction in human pluripotent stem cells and thus recapitulate stages of embryogenesis synthetically. Moreover, we report high resolution force maps in three dimensions that depict and quantify the effects of apical contractility, proving that it generates crucial out-of-plane deformations as in many morphogenetic events. In addition, we prove that the consequences of such localized cell shape change are long-range transmitted and generate tissue scale effects, coherent with *in vivo* situations. Since most of the state-of-the-art organoid technology relies on stem cells, the mechanical insights we obtain from controlling the forces of apical constriction are applicable to a wide variety of systems. We anticipate our study will establish a physical quantification of this cell shape change and determine its relevance in different embryo and organogenesis contexts.

FLASH presented by:

NAME: Amélie Godeau
GROUP: Bioengineering in reproductive health
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Mechanics Of Mouse and Human Embryo Implantation

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During implantation, the mammalian embryo attaches to the endometrium, the tissue lining the mother uterus. The mural trophoblast differentiates into giant trophoplast cells which invade the collagen-rich tissue of the endometrium while the epiblast and the extraembryonic ectoderm establish the proximal-distal and the anterior-posterior axis thus breaking the spherical symmetry of the embryo. Here we develop a novel ex-vivo method to perform traction force microscopy on extracellular matrix to reveal forces applied by embryos. Mouse embryos remodel the collagen matrix while implanting, creating a rim of collagen. They show anisotropic radial displacement of collagen on various displacement axes with traction fluctuations over time. However, remarkable differences exist between the mouse and the human embryos. Human embryos before implanting, embed themselves in the collagen matrix by sinking in and exert forces isotopically. In addition, for both species pairwise embryos can mechanically interact, by applying a force directed toward each other and collagen densification can be observed. The embryos, sense and react to external force cues such as spheroids or collagen pulling by a microneedle. This leads to an alignment of the outgrowth or of the proximal-distal embryo axis in mouse and triggers myosin enrichment and directional growth of a cell projection for human embryos. Altogether, our method allows for a better understanding of the role of mechanical forces during embryo implantation and reveals mechanosensitive behaviour of embryos.

FLASH PRESENTATION | MECHANOBIOLOGY

FLASH presented by:

NAME: Ignasi Granero
GROUP: Cellular and molecular mechanobiology
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Development of a sensor to study mechanotransduction in the nucleus

Ignasi Granero-Moya [1], Ion Andreu [1], Guillaume Belthier [2], Bart Groenen [1], Marc Molina Jordán [1], Miguel González Martín [1], Jacco van Rheenen [2], Pere Roca-Cusachs [1,3]
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Environmental and cellular forces affect cell behavior, homeostasis, and development. Mechanotransduction happens when cells transduce these forces into biochemical signals, which in turn regulate transcription. To understand how this happens in the eukaryotic nucleus, we have designed and tested a sensor of nuclear mechanotransduction , based on our finding that active transport between the cytoplasm and the nucleus depends on force. The sensor is a fluorescent protein undergoing active transport into the nucleus, which changes its nuclear concentration when force is applied to the nucleus. The readout of the sensor is the nuclear to cytoplasmic ratio of the fluorescence emitted by the protein. Upon forces reaching the nucleus, the sensor translocates to the nucleus and the nuclear to cytoplasmic ratio increases. For understanding this phenomenon, we have automatically computed sensor ratio, cell density, nuclear shape parameters, nuclear size. We see changes in sensor ratio when using a multicellular approach with epithelial and mesenchymal cell monolayers, where cells are affected by forces reaching the nucleus. As a complimentary approach to alter forces reaching the cell nucleus, cells have been submitted to osmotic shocks and contractility inhibition, also showing effect on nuclear-to-cytoplasmic transport.

FLASH presented by:

NAME: Jorge Oliver-De La Cruz
GROUP: Cellular and molecular mechanobiology
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Exploring neuronal mechanosensing using an optimized cellular model

Oliver-De La Cruz, Jorge [1], Hinojosa Grajales, Yara [1], Viswanadha, Srivastava [1], Roca-Cusachs, Pere [1,2]
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Neurodegenerative disorders, such as Alzheimer's disease, present a significant social challenge worldwide, particularly due to aging populations. The ultimate causes behind the pathological events leading to neuronal loss in these disorders remain unidentified, despite extensive research efforts. Interestingly, most of these diseases are associated with changes in the mechanical properties of the brain. Although the ability of different cell types to perceive and adjust to mechanical signals is well understood, studies focused on mechanotransduction in adult neurons are very limited.

Therefore, we decided to explore neuronal mechanosensing by developing an optimized model based on SH-SY5Y neuron-like cells. Our new novel protocol, which combined biochemical induction and genetic control of the master gene NGN2 expression, allows the SH-SY5Y cells to differentiate into neuron-like cells with a more mature phenotype in a shorter period. Subsequently, these neuronal-like cells were exposed to various matrix rigidities, confirming their ability to sense and adapt their morphology to the environmental stiffness. Furthermore, we observed detectable levels of the co-transcriptional factor YAP, and for the first time, we ruled out that its nuclear presence is mechanoregulated in neuronal cells. We then utilized this system to investigate changes in TAU distribution in response to alterations in matrix compliance. Finally, we validated these results using a more advanced model of hiPSC-derived neurons.

In conclusion, our study offers a novel approach to investigating the mechanobiology of neurodegenerative diseases by integrating improved differentiation methods, genetic modification, and mechanical stimulation. This model holds the potential to uncover underlying molecular mechanisms, paving the way for innovative therapeutic strategies in the future.

FLASH PRESENTATION | ICT FOR HEALTH

FLASH presented by:

NAME: Eduardo Caballero Saldivar
GROUP: Signal and information processing for sensing systems
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Processing of Gas Chromatography Ion Mobility Spectrometry data for urine Colorectal cancer metabolomics

S. Oller-Moreno [1,2], L. Fernández [1,2], E. Caballero [1], C Mallafré-Muro [1,2], A. Pardo [2], S. Marco [1,2].
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Gas chromatograph coupled with Ion mobility Spectrometry (GC-IMS) has emerged as a powerful tool to analyse volatile chemical matrices because of its high sensitivity and speed. GC-IMS has been wieldy used in the sectors of security and food industry and recently some biomedical applications have been explored analysing Volatile Organic Compounds (VOCs) generated by biofluids. Despite of the advantages that the GC-IMS offers there are still some drawbacks, the data obtained is highly dimensional and affected by uncontrolled environmental changes such as temperature and humidity, among others. To overcome these problems and have good quality data to apply the Machine Learning algorithms some pre-processing steps such as smoothing and alignment among other are needed.

We developed an R Package to deal with these problems and to present a user-friendly workflow to deal with this kind of data and go from the raw data to a data matrix containing relevant information to do the further classification of samples. This R package was published in GitHub for everyone to be able to use it. The workflow proposed to analyse the samples is as follows: First import the raw data, then apply the required pre-processing steps (cutting, smoothing, alignment and decimation), extract the information of the sample identifying the peaks (compounds) and matching them across samples and finally estimate the baseline and integrate the area of each peak to create the final data matrix.

To test this package urine samples were taken from 29 subjects, 15 with Colorectal cancer (CRC) and 14 controls; the samples were analysed in the with the GC-IMS FlavourSpec from GAS Dortmund, the resulting files were imported to R and processed obtaining a final data matrix in which every row represented a sample and every column a detected peak (compound); with this data matrix Machine learning models can be applied to discriminate the samples as healthy and CRC. It is expected that this will provide an accurate and non-invasive alternative to do early diagnostics of CRC, avoiding invasive diagnostic techniques such as colonoscopy and ultimately reducing the mortality rates of CRC, that in 2020 represented 9.4% of cancer related deaths.

FLASH presented by:

NAME: Eva Martín
GROUP: Signal and information processing for sensing systems
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Fusing Clinical and Metabolomic Data for the Prediction of Ventilatory Therapies in ICU COVID Patients

S.Oller Moreno [1,2], E.Martín1, P.Nebot [3*], FJ. Parrilla-Gómez [3,4], A. Castellví-Font [3,4], P. Pérez-Terán [3,4,5], A. Pardo [2], J. Masclans [4], O. Pozo [3], S. Marco [1,2]*

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The respiratory clinical evolution of COVID-19 remains poorly understood. Exploring the ICU patient's clinical variables, as well as the altered metabolic patterns associated with the disease, is crucial for better comprehending COVID-19's pathophysiology. In line with this objective, this project aims to find a way to make accurate respiratory prognoses for severe COVID-19 patients by utilizing clinical and metabolomic data. When severely affected patients with COVID-19 are admitted to the UCI, they often require non-invasive ventilatory support systems (NIS), such as High flow oxygenation, CPAP and BiPAP. While some patients respond positively to the ventilatory support and improve their condition, others do not and require intubation. An early identification of patients who may require intubation is vital, as early intubation is preferable if the non-invasive ventilatory support system is ineffective. Predicting the failure of the ventilatory support system could potentially improve the patient's outcome by facilitating timely interventions. Statistical and artificial intelligence techniques were used to develop a predictive model that accurately identifies patients at risk of ventilatory support failure, signaling the need for intubation. The study includes the assessment of a wide range of clinical variables, such as sex, age, comorbidities, oxygen saturation levels, and respiratory rate, in conjunction with metabolomic data collected at different time points after the initiation of ventilatory support. The predictive model demonstrated a good performance in distinguishing patients who would require intubation from those who would not, with an AUC value of 0.73 (95% CI, 0.66 to 0.81). Moreover, among the clinical and metabolomic variables under study, Apache II and octanoic acid emerged as the most significant predictors, in combination with many other variables such as the SaFi index or the isovaleric acid. Ultimately, based on these significant variables the goal is to establish a decision algorithm that guides clinical diagnosis and improves the overall care of COVID-19 patients.

FLASH PRESENTATION | NANOMEDICINE

FLASH presented by:

NAME: Víctor Campo Pérez
GROUP: Bacterial infections: antimicrobial therapies
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Mycobacterium abscessus and *Pseudomonas aeruginosa* cooperate to evade immune response in the context of cystic fibrosis

Víctor Campo-Pérez [1,2], Esther Julián [2,] and Eduard Torrents [1,3,*]*
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The incidence of infection by nontuberculous mycobacteria, mainly Mycobacterium abscessus, in patients with cystic fibrosis and other chronic pulmonary illnesses is increasing, translating into an acceleration in the decline of lung function. In most cases, *M. abscessus* coinfects with *Pseudomonas aeruginosa*, the most common pathogen in these chronic diseases. However, it is unknown how these two bacterial species interact when coinfecting. This study aims to explore the behavior of both species in three relevant pathogenic settings: dual-species biofilm development using a recently developed method to monitor individual species in dual-species biofilms; coinfection in bronchial epithelial cells using *in vitro* assays; and *in vivo* coinfection using the Galleria mellonella model. The results demonstrate the capability of both species to form stable mixed biofilms and to reciprocally inhibit single-biofilm progression. Coinfections in bronchial epithelial cells were correlated with significantly decreased cell viability, while in G. mellonella, coinfections induced lower survival rates than individual infections. Outstandingly, the analysis of the immune response triggered by each bacterium in bronchial epithelial cell assays and G. mellonella larvae revealed that *P. aeruginosa* induces the overexpression of proinflammatory and melanization cascade responses, respectively. In contrast, *M. abscessus* and *P. aeruginosa* coinfection significantly inhibited the immune response in both models, resulting in worse consequences for the host than those generated by single *P. aeruginosa* infection. Overall, the presence of *M. abscessus* produces a decline in the immune responses that worsens the infection and compromises the host.

FLASH presented by:

NAME: Antonino Nicolò Fallica
GROUP: Nanomalaria
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Targeting protein aggregation with bis(styrylpyridinium) salt derivatives: design, synthesis and preliminary biological evaluation of novel and promising antimalarial drugs

A.N. Fallica [1,2,3], M. Ramírez [1], L. Román-Álamo [1,2,3], C. Camarero-Hoyos [1,2,3], Y. Avalos-Padilla [1,2,3], E.M. Arce [4,5], P. Reolid [4,5], A. Mallo-Abreu [4,5], D. Muñoz-Torrero [4,5], X. Fernàndez-Busquets [1,2,3]
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Malaria is a highly life-threatening infectious disease caused by parasites of the genus Plasmodium. The emergence and spread of drug resistance phenomena to the current antimalarial chemotherapeutics contribute to its high mortality rate worldwide. Therefore, the identification of compounds that act with a novel mechanism of action and simultaneously prevent the selection of resistant mutants is urgently needed. Of interest, the *Plasmodium falciparum* proteome is rich in long glutamine/asparagine repeats which are responsible for the occurrence of protein aggregation. In light of this, our research group has recently investigated the functional significance of protein aggregation in *Plasmodium falciparum* [1]. Interestingly, we discovered that YAT2150, a bis(styrylpyridinium) salt contained in the commercial protein aggregation detection reagent Proteostat®, is a potent protein aggregation inhibitor endowed with a fast-acting antimalarial activity in both asexual and sexual parasitic blood stages and it is also active against artemisinin- and chloroquine-resistant lines. However, the observed cytotoxicity and low selectivity index could slow down YAT2150 investigation for additional preclinical studies. On these grounds, we herein report the design, synthesis and preliminary biological evaluation of novel YAT2150 derivatives. As the parent compound, the novel analogues are characterized by an easy, fast and inexpensive synthesis. Chemical modifications to the parent compound allowed us to define structure-activity relationships for this novel class of antiplasmodial agents. Among the new 17 synthesized compounds, some of them displayed a more potent growth inhibition potency and lower cytotoxicity when compared to YAT2150, with consequent better selectivity index ratios. Further details will be discussed at the meeting.

FLASH PRESENTATION | NANOMEDICINE

FLASH presented by:

NAME: Sujey Palma Florez
GROUP: Nanobioengineering
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Human cortical neurons incorporation into a microfluidic model for drug screening in neurodegenerative diseases

Palma-Florez Sujey [1], Palma-Tortosa Sara [2], Kokaia Zaal [2], Samitier Josep [3,1,4] , Lagunas Anna [3,1], Mir Mònica [3,1,4]
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Most of the neurodegenerative diseases (NDDs) are characterized by the degeneration of the neurons in the nervous system. Neurofilament light chain (NfL) is a promising biomarker for monitoring NDDs because it is a protein that is exclusively expressed in neurons and is only released upon neuronal damage. The development of *in vitro* models with human neurons could offer a platform to evaluate the performance of a drug against neurodegeneration. In this work, we optimized the incorporation of cortical neurons derived from long-term neuroepithelial-like stem (lt-NES) cells in a microfluidic device. Lt-NES cells are produced from dermal fibroblasts which are subjected to retroviral transduction to obtain human-induced pluripotent stem cells (iPSCs) and later induced to differentiate into a neural phenotype through an embryoid body-production step. Neural rosettes are generated and carefully selected to isolate clusters and grown in the presence of several neuronal promoting factors resulting into the lt-NES cell line. To accomplish our goal, we first performed a differentiation protocol based on small molecules strategy to obtain cortical progenitors from lt-NES cells. Then, the cortical progenitors were embedded in different 3D scaffolds and injected into the chip. Bright field images were taken to determine cell survival and morphology. In addition, neuronal differentiation was evaluated into the chip using immunofluorescence with neuronal markers such as Tuj-1, MAP2 and NfL. Furthermore, cortical neurons were co-cultured with human astrocytes and pericytes. In addition, to promote NfL release by axonal degeneration for later quantification with a biosensor, we optimized the neuronal death by excitotoxicity using N-methyl-D-aspartate (NMDA) at different concentrations. Our results showed that Matrigel is an optimal scaffold for the survival of neural progenitor and allows their differentiation into mature neurons and visualization of their projections. In addition, cortical neurons embedded in Matrigel expressed Tuj-1, MAP2 and NfL after 7 and 14 days into the microfluidic devices. Incubation with NMDA at different concentrations (1, 5 and 10 μM) for 30 min showed neuronal death after 24 hours presenting a dose-dependent effect. Also, we will monitor the NfL release after NMDA administration in our microfluidic device.

FLASH presented by:

NAME: Meritxell Serra-Casablancas
GROUP: Smart nano-bio-devices
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Radionuclide therapy with accumulated urease-powered nanobots reduces bladder tumor size in an orthotopic murine model

Cristina Simó [1#], Meritxell Serra-Casablancas [2#], Ana C. Hortelao [2], Valerio Di Carlo [2], Sandra Guallar-Garrido [3], Sandra Plaza-García [1], Pedro Ramos-Cabrer [1,4], Balbino Yagüe [1], Laura Aguado [1,5], Lídia Bardia [6], Sébastien Tosi [6], Vanessa Gómez-Vallejo[1], Abraham Martín [4,5], Tania Patiño [2,7], Esther Julián [3], Julien Colombelli [6], Jordi Llop [1*], Samuel Sánchez [2,8*]*

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Bladder cancer (BC) is among the most common cancers worldwide. Current treatment methods involving intravesical drug administration present good survival rates but low therapeutic efficacy. Self-propelled nanoparticles (nanobots) could improve efficacy through their enhanced diffusion and mixing capabilities in urine compared to conventional drugs or passive nanoparticles. Here, we tested radiolabeled mesoporous silica-based urease-powered nanobots in an orthotopic murine model of BC. *In vivo* and ex vivo results demonstrate enhanced accumulation of nanobots at the tumor site. Positron emission tomography revealed an 8-fold increase in accumulation *in vivo*. Inductively coupled plasma mass spectrometry confirmed these results. A custom label-free optical contrast based on polarization-dependent scattered light-sheet microscopy was applied to cleared bladders to confirm tumor penetration by nanobots. Treating tumor-bearing mice with intravesically administered radio-iodinated nanobots for radionuclide therapy resulted in tumor size reductions of about 90% compared with non-treated mice, positioning nanobots as efficient delivery nanosystems for BC therapy.

FLASH PRESENTATION | NANOMEDICINE

FLASH presented by:

NAME: Lena Witzdam
GROUP: Bioinspired onteractive materials and protocellular systems
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Interactive coatings direct blood components to prevent clot formation in blood-contacting medical devices

Witzdam, Lena [1,2,3]; Obstals, Fabian [2,3]; Garay-Sarmiento, Manuela [1,2,4]; Quandt, Jonas [2,3]; Kostina, Nina [1]; Grottke, Oliver [5]; Rodriguez-Emmenegger, Cesar [1,2,6,7]
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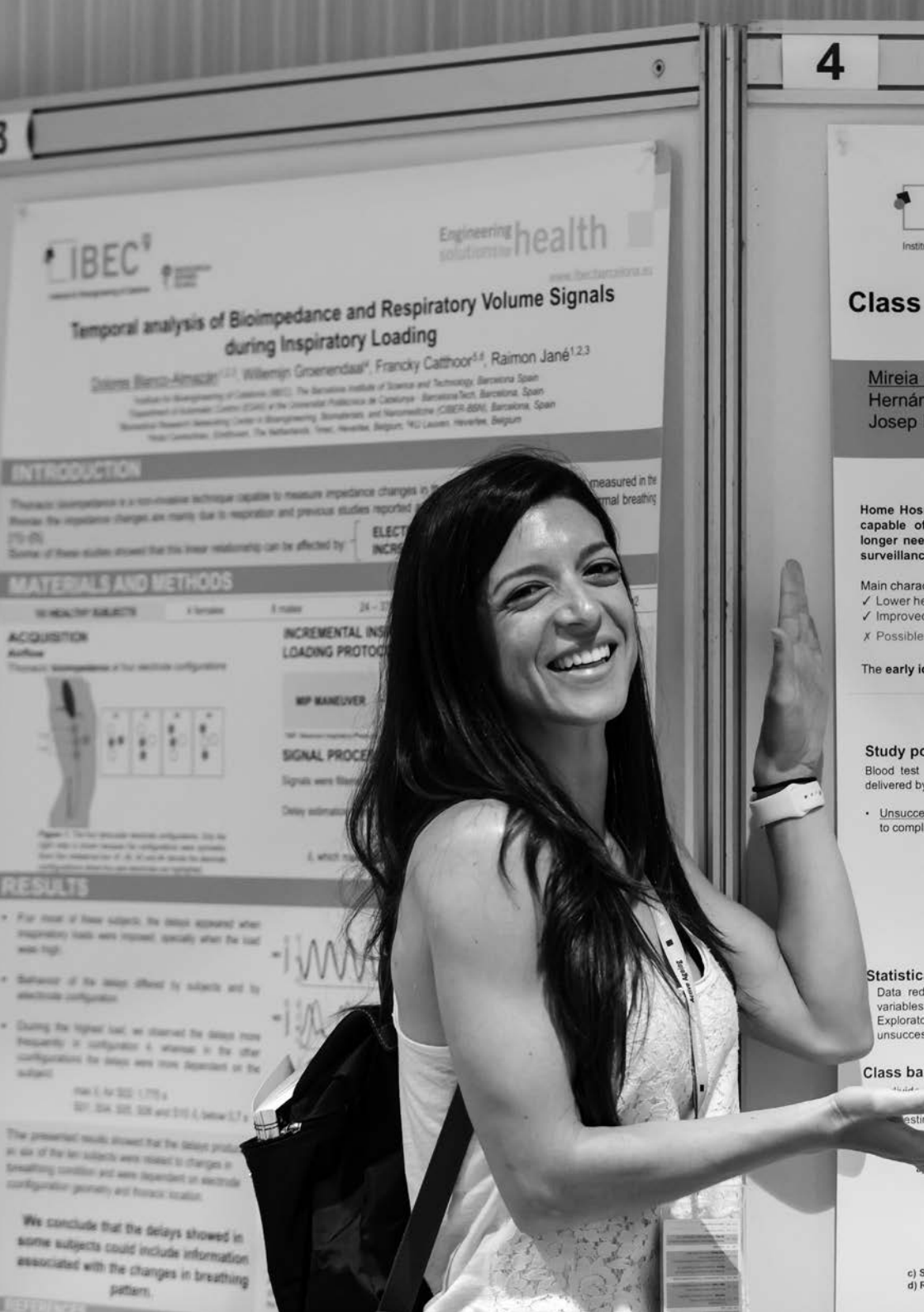
In blood-contacting medical devices, the contact of blood with the artificial surface inevitably causes the activation of coagulation. Immediately after the contact of blood with the surface, protein adsorption occurs. This leads to the reciprocal activation of factor XII and plasma prekallikrein generating large amounts of thrombin resulting in clot formation. Thus, the use of blood-contacting medical devices can lead to life-threatening complications such as thrombosis and stroke. In nature, the lining of healthy endothelium can sense and maintain a tightly regulated equilibrium called hemostasis that prevents hemorrhages and excessive coagulation. Our goal is to develop coatings inspired by the endothelium that turn the surface of medical devices hemocompatible to prolong their use without negative outcomes. Towards this aim, we develop coatings that go beyond passivation by interacting with blood components and orchestrate a cascade of reactions to enhance their hemocompatibility and performance. The coatings include three hierarchical levels: a passive, a modulatory, and an interactive one. The passive level consists of antifouling polymer brushes that create a physical barrier to protein adsorption and cell adhesion, prohibiting surface-induced coagulation activation. The modulatory level is achieved by decorating the brushes with small biomolecules capable of binding to key elements of the coagulation cascade and inactivating them directly at the surface of the device. In contrast to anticoagulants, this approach allows a local inhibitory effect at the surface and does not interfere with hemostasis. The interactive level can sense the presence of a thrombus formed somewhere else in the system and orchestrates its disintegration. We developed a fibrinolytic coating that is only active in the presence of a thrombus and directs its destruction using components present in the blood. We envision that our coatings are a promising route toward the improvement of the hemocompatibility of blood-contacting medical devices.


4
IBEC
Engineering health
Temporal analysis of Bioimpedance and Respiratory Volume Signals during Inspiratory Loading
Willemijn Groenendaal, Francky Catthoor, Raimon Jané
INTRODUCTION
MATERIALS AND METHODS
ACQUISITION
INCREMENTAL
LOADING PROTOC
MIP MANEUVER
SIGNAL PROCE
RESULTS
We conclude that the delays showed in some subjects could include information associated with the changes in breathing pattern.
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Imbalance Impact on the Prediction of Complications during Home Hospitalization: A Comparative Study

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Introduction

pitalization (HH) is presented as a healthcare alternative f providing high standards of care when patients no d hospital facilities, but still require active and complex e [1].

cteristics of home hospitalization programs:
ealthcare-associated costs
d patient's quality of life
complications due to lack of continuous observation at home

dentification 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

opulation

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

ssful group: 101 patients eventually needing regular hospitalization due ications of different origin.

	Successful (n=1850)	Unsuccessful (n=101)	p-value
Age, *years old*	70.6 ± 15.0	72.9 ± 14.7	0.072
Male sex, *n* (%)	1153 (62.3%)	66 (65.3%)	0.613
Main diagnosis, *n* (%)			
Cardiology	196 (10.6%)	26 (25.7%)	<0.001
Respiratory	573 (31.0%)	24 (23.8%)	0.156
Oncology	146 (7.9%)	8 (7.9%)	1.000
Surgery	366 (19.8%)	15 (14.9%)	0.276
Acute	569 (30.8%)	28 (27.7%)	0.594

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

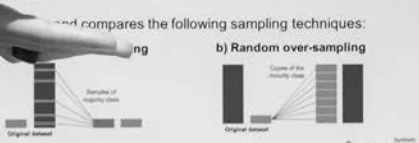
al analysis

lundancy evaluation through **correlation analysis** between pairs of (*Spearman*).
ry analysis of **statistical differences** between successful and ssful cases for each variable (*Mann-Whitney U tests*).

lancing

ining (75%) and testing (25%) subsets, using a stratified strategy. model training were only applied to the first subset.
g subset was then used for classification performance quantification.

nd compares the following sampling techniques:



ynthetic Minority Over-sampling TEchnique (SMOTE)
Random Over-Sampling Examples (ROSE)

Results

Correlation analysis

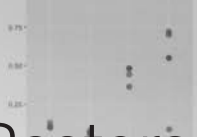
- **Hematocrit** was positively correlated with both **hemoglobin concentration** ($\rho = 0.98$) and **red blood cell count** ($\rho = 0.91$).
- Percentage and total amount of **neutrophils** ($\rho = -0.97$) and **lymphocytes** ($\rho = 0.96$) were highly correlated.

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 *Sensitvity*: ROSE
  - Best F_1: Random over-sampling



Posters

Conclusions

I. Significant correlations were noted among variables. Thus, a **feature selection** step would be advisable to minimize data redundancy.
II. **Hemoglobin concentration**, **lymphocytes**, **red cell distribution width** and **creatinine** were found to unmask statistically significant differences between patients undergoing successful and unsuccessful HH stays.
III. 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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2	Marta	Badia Graset	A minimal model for the formation of non-toxic protein aggregates by TDP-43
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POSTER 1 presented by:

NAME: Gaia Amato
GROUP: Pluripotency for organ regeneration
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

3D models to study CAKUT disease: from CRISPR/Cas9-engineered hPSC lines to the differentiation of human kidney organoids.

Gaia Amato [1], Carolina Tarantino [1], Daniel Moya-Rull [1], Andrés Marco [1], Elena Garreta [1], Nuria Monsterrat [1,2,3]
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Congenital anomalies of the kidney and urinary tract (CAKUT) are a group of malformations that can arise during embryonic development in the kidneys, the urethra, the ureters, and the bladder. It is estimated that 4-60 per 10.000 births are diagnosed with some form of CAKUT and, nowadays, only invasive treatments such as urologic surgery, dialysis or transplantation represent a therapeutic alternative. Approximately only 20% of the cases of CAKUT can be attributed to genetic mutations. Interestingly, PAX2 and HNF1B are commonly included in diagnostic screening due to their high frequency of detection as mutated genes. Importantly, challenges remain with regards to the identification of new treatments. Human kidney organoids have emerged as an essential tool in studying CAKUT disease gestation and progression with the goal to be used as disease modelling cell culture platforms. To date, most of the works focused on CAKUT disease modelling using kidney organoid relay on the use of human induced pluripotent stem cells (hiPSCs) as primary cell source, with only few studies taking advantage of genome editing in human pluripotent stem cells (hPSCs) to mirror CAKUT genetic background(s). In our laboratory, we have CRISPR/Cas9 to engineer hPSCs and create reporter and knock out (KO) lines to model CAKUT disease.

Making use of our recently developed iC2 platform, we have either introduced a fluorescent protein or induced a double strand break for the generation of reporter cell lines (to monitor the expression and localization of the gene/protein of interest) and knock out lines (to disrupt protein expression), respectively. Our approach has allowed for the generation of GATA3-mOrange and MEIS1-mPlum reporter cell lines, which have been validated at the pluripotent and kidney organoid stages. In order to further understand the impact of PAX2 and HNF1B mutations in the GATA3 lineage, we have also generated PAX2 and HNF1B KO in the background of the GATA3-mOrange reporter cell line. At the present time these cell lines are being characterized for the successful suppression of the target proteins (Western blot and confocal microscopy analysis). Furthermore, experiments making use of our recently developed procedures for the generation of nephron-like kidney organoids will allow us to dissect the function of these genes during nephron progenitor derivation and nephron induction. Overall, through the execution of these experiments we will generate an isogenic platform allowing for the interrogation of renal and extra-renal manifestations due to PAX2 and HNF1B also differentiating the lines generated here towards other organoid models currently available in the laboratory, including heart and retina.

POSTER 2 presented by:

NAME: Marta Badia Graset
GROUP: Protein phase transitions in health and disease
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

A minimal model for the formation of non-toxic protein aggregates by TDP-43

Marta Badia, Benedetta Bolognesi
[1] *Institute for Bioengineering of Catalonia*

Protein aggregates are a prominent feature of many neurodegenerative diseases. For example, in all cases of Amyotrophic lateral sclerosis (ALS), aggregates of the protein TDP-43 are found in patients motor neurons. However, the relationship between TDP-43 aggregation and toxicity remains unclear. Growing evidence suggests that TDP-43 toxicity is linked to abnormal protein condensation, rather than aggregation. Using deep mutational scanning (DMS), our lab has previously shown that mutations to hydrophobic residues in TDP-43 prion-like domain (PLD) are less toxic and lead to the formation of solid-like aggregates.

Here, we employed an assay that directly examines the initial stages of protein aggregation and identified a specific region (residues 321-343) within TDP-43 PLD that rapidly forms insoluble aggregates. This region corresponds to an evolutionary conserved segment of TDP-43 PLD, a segment which we previously showed to be a hotspot of toxicity, as amino acid substitutions in this region had the largest detrimental or protective effects on cell viability. By quantifying the impact of hundreds of single mutants on the aggregation propensity of this region, we find that mutations that increase hydrophobicity, particularly those involving changes to aromatic residues, strongly enhance aggregation. What is more, inside this aggregation hotspot, we find a stretch where mutational impact suggests the formation of two beta-strands whose pairing is required for aggregation to proceed.

We hypothesize that this type of aggregation may represent a way to divert TDP-43 away from more toxic states. Additionally, we observed a correlation between the mutational signature in this aggregation dataset and multiple DMS datasets, suggesting that this could be a more generalized aggregation pathway that various proteins can undergo without resulting in toxicity.

POSTER 3 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 cancer

David Bartolomé-Català [1], Jordi Comelles [1], María García-Díaz [1,3] and 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).
[2] Centro de Investigación Biomédica en Red (CIBER).
[3] Department of Electronics and Biomedical Engineering, University of Barcelona (UB).

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 (1). 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. However, studying this process *in vivo* is extremely difficult and costly, and standard *in vitro* models are too simplistic to fully recapitulate the journey of T cells from the blood stream to the tumor. Recent advances in biofabrication such as 3D bioprinting have opened new avenues to obtain three-dimensional (3D) *in vitro* models mimicking the *in vivo* TME. In this work, we have used *in vitro* models of increasing complexity to study the transendothelial migration (TEM) and T cell motion though the stroma: Transwell models (i) coated with adhesion molecules (iCAM1 or Fibronectin) or (ii) cultured with HUVEC cells mimicking the endothelial barrier, and (iii) a novel bioprinted model that recapitulated both the endothelial barrier and the stromal compartment.

Jurkat T cells were used to validate both the standard microporous membrane coated with adhesion molecules and with the endothelial monolayer. Strikingly, a chemotactic stimulus enhanced transmigration in both scenarios. Finally, we used a bioprinted model based on gelatin methacrylate (GelMA) and polyethylene glycol diacrylate (PEGDA) with embedded intestinal fibroblasts to mimic the stromal component of the TME. On top of this hydrogel, we seeded HUVEC cells forming the endothelial barrier that T lymphocytes encounter when accessing the tumor. Preliminary data suggest that Jurkat cells were unable to cross endothelial barrier and navigate the stromal compartment even in the presence of chemotactic stimulus. Further experiments to assess the role of GelMA-PEGDA composition and cell type in the impairment of Jurkat's migration were performed. On the one hand, we observed that primary mouse CD8 T cells were able to migrate in collagen hydrogels, but not in GelMA-PEGDA. On the other hand, Jurkat cells were not able to migrate in none of the hydrogels tested. Therefore, both the cellular model and the hydrogel's physico-chemical properties need to be further adjusted to retrieve the physiological dynamics of T cells in the TME. These results suggest that the primary T cells are a more representative model for the study of T cell migration in 3D matrices, and the importance of having more realistic platforms than standard Transwell assays to study T cell migration.

POSTER 4 presented by:

NAME: Armando Cortés Reséndiz
GROUP: Biosensors for bioengineering
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Unraveling the NASH-Muscle Atrophy Connection: Functional Analysis of Sarcopenic Skeletal Muscle *in vitro*

Cortés-Reséndiz, Armando [1], De Chiara, Francesco, [1], Fernández-Garibay, Xiomara [1], Mughal, Sheeza [1], Fernández-Costa, Juanma [1], Ramón-Azcón, Javier [1,2]
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[2] Catalan Institution for Research and Advanced Studies (ICREA), Spain

Sarcopenia, a condition characterized by skeletal muscle (SM) insufficiency and failure, significantly impacts the mobility and overall independence of elderly patients. Recent studies have revealed a mutually detrimental relationship between sarcopenia and non-alcoholic steatohepatitis (NASH), a progressive form of fatty liver disease. Sarcopenia not only increases the risk of developing NASH and its complications but is also worsened by the presence of NASH. This complex interplay between the two conditions leads to heightened patient frailty and exacerbates liver transplantation outcomes. Reduced SM strength and quality coincide with the onset and progression of NASH. Therefore, understanding the molecular associations between NASH and sarcopenia is crucial for identifying potential drug targets, and developing targeted therapeutics.

This study aims to investigate the bidirectional effects of NASH and sarcopenia using a cutting-edge organs-on-chip (OOC) platform. OOC technology offers an alternative to traditional 2D culture systems by faithfully replicating cellular microenvironments, and tissue architecture.

We hypothesize that by mimicking the NASH-sarcopenia relationship in this model, we can gain insights into disease mechanisms and facilitate the development of targeted therapeutics. We will examine the impact of NASH on human differentiated myotubes within a 3D setting. Building upon previous studies in murine cell lines, we anticipate an atrophic phenotype in the myotubes because of NASH-induced changes such as steatosis and activation of hepatic stellate cells (HSCs).

To create our NASH model, we employed an OOC approach. Human hepatocytes and HSCs were encapsulated in a collagen-based hydrogel, significantly replicating hepatic steatosis, and facilitating HSC activation, the primary driver of liver fibrosis. The model was challenged with non-esterified fatty acids (NEFAs) and LPS to achieve the desired phenotypes. Additionally, we utilized 3D human differentiated myotubes, subjecting them to the environment generated by the NASH model to evaluate its functional impact on SM cells, expecting an atrophic phenotype.

This study aims to establish a foundation for further investigations into the associations between NASH and sarcopenia. The use of OOC technology poses an opportunity to gain insights into the interactions between different organs, accelerating the identification of potential therapeutic targets for these interconnected diseases.

POSTER 5 presented by:

NAME: Judith Fuentes Llanos
GROUP: Smart nano-bio-devices
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Integration of ferrofluid into 3D bioprinted skeletal muscle-based actuators provides magnetic guidance and improves force contraction

Judith Fuentes [1†], Zoran M. Cenev [2†], Maria Guix1*, Anna Bakenecker [1], Noelia Ruiz [1], Grégory Beaune [2], Pedro Ramos Cabrer [1], Jaakko V.I. Timonen [2], Samuel Sanchez [1], Veronika Magdanz [1,3*]*
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Three dimensional bioprinting has opened new possibilities for the bioengineering of skeletal muscle models with organization and functionality similar to native tissues. This is key to understand the physiological conditions of skeletal muscle to integrate some of their unique properties, such as self-healing, adaptability, and response to external stimuli, in biohybrid systems. However, incorporating additional functionality while maintaining biocompatibility and degradation control is still a challenge.

In this work, we developed a 3D bioprinted skeletal muscle-based actuator which is magnetically active due to the integration of ferrofluid, a water-based solution comprising citrated iron oxide nanoparticles, in the cell-laden bioink. This magnetic tissue, named here as ferromuscle, is characterized by improved tissue differentiation, demonstrated by increased force output upon electrical stimulation. The magnetic component arising from the ferrofluid allows the magnetic guidance of the bioactuators and promotes integration and cell migration between co-cultured muscle tissues, which is an interesting aspect for regenerative medicine purposes. Moreover, the integrated ferrofluid can act as a contrast agent for magnetic resonance imaging. Further, cytocompatibility and degradation of the ferromuscle over time are also demonstrated. These results offer a new way to position and monitor future muscle implants.

POSTER 6 presented by:

NAME: Manuela Garay-Sarmiento
GROUP: Bioinspired onteractive materials and protocellular systems
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Designing zwitterionic granular hydrogels towards specific interactions with living matter

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Granular hydrogels have emerged as a promising biomaterial platform for *in vivo* tissue repair and *in vitro* cell culture. They are formed by the packing and subsequent crosslinking of hydrogel microparticles (microgels). Owing to their jammed structure, granular hydrogels feature a three-dimensional interconnected porous network that allows cell migration, proliferation and mass transport within the hydrogel. Furthermore, they have shear-thinning and self-healing properties that render them ideal for injection, extrusion and 3D printing applications, enabling constructs that better mimic the complexity and heterogeneity of native tissues. In this poster we present a new family of interactive granular hydrogels composed of zwitterionic poly(carboxybetaine) polymers (pCB). pCB yields microgels that are superhydrophilic, non-immunogenic and resistant to protein fouling. This creates a basis for protection against unwanted interactions with living matter, such as cells and pathogens. Interactive properties are then achieved by functionalizing the pCB microgel surface with selective biomolecules that convey specific signaling cues. We prepared a diverse set of microgels in the scale of 60-300 _m using three different fabrication techniques - batch emulsion, microfluidics, and mechanical extrusion fragmentation. Furthermore, we achieved microgels with storage moduli ranging from ~10-10,000 Pa, which span the stiffness regime of mammalian soft tissue and cartilage. Microgels could be cross-linked by covalent (enzymatic and phototriggered reactions) and reversible non-covalent linkages to form pCB granular hydrogels with porosities of _8-30% and excellent cell compatibility. The modular nature and ease of functionalization of our pCB granular hydrogels facilitates customization to target distinct applications in tissue engineering, sensing, and living therapeutics.

We envision that our work may contribute to the next generation of specific cell-instructive granular materials capable of directing cell behavior in a superior-manner.

POSTER 7 presented by:

NAME: Amayra Hernandez Vega
GROUP: Molecular and cellular neurobiotechnology
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Tau Dynamics and Cooperative Interactions: possible relevance for its role as a Microtubule Associated Protein and for its pathological Solid Transition in Neurodegenerative Diseases

Amayra Hernández-Vega [1,2,3], Marcus Braun [4], Valerie Siahaan [4], Jochen Krattenmacher [4], Zdenek Lansky [4], Lara Scharrel [5], Marcus Jahnel [6], Susanne Wegmann 7, Bradley T Hyman [8], Jose Antonio Del Rio [1,2,3,9], Simon Alberti [10], Stefan Diez [5,6], Anthony A Hyman [11]
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[8] Department Neurology, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, USA.
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[11] Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

Tau is an intrinsically disordered protein with multiple binding sites for tubulin and a stereotyped blocky distribution of charges along its length. These characteristics prompted us to investigate if Tau could form molecular condensates and, if so, if this was relevant for its biological function or pathological solid transition. Using recombinant Tau, we found that, under a crowded environment, Tau can undergo liquid-liquid phase separation. These drops are sensitive to ionic strength suggesting that electrostatic interactions are involved in Tau drop formation. In line with this, we showed that both RNA and tubulin enhance their formation. Further, we found that Tau condensates concentrate tubulin and locally nucleate microtubule (MT) bundles. This process is reversible and, by outcompeting MT binding with heparin, we could reverse Tau into a drop-like configuration and unbundled MTs. In a following study, we found that MTs could serve as a platform to nucleate Tau dynamic cooperative assemblies. These structures form in the absence of molecular crowding and at more relevant biological concentrations of Tau. Like condensates, these structures are in equilibrium with Tau in solution, however, unlike them, they are composed of a single layer of Tau. This suggests that in-cis interactions between Tau molecules are favored in this configuration. Further,

we showed that these structures shield MTs from katanin 2 severing action and interfere with motor displacement. In parallel, we have investigated conditions/co-factors that enhance Tau solid transitions. We are now applying the knowledge obtained from this work to: (i) understand how Tau transitions from an axonal Microtubule Associated Protein into solid pathological structures in Alzheimer´s Disease; and (ii) investigate the immediate consequences of this transition for axonal transport and overall neuronal integrity. To this end, we combine *in vitro* reconstitution with neurons in culture (hPSC-derived) using a live cell imaging approach.

POSTER 8 presented by:

NAME: Dayaneth Jácome Montero
GROUP: Molecular and cellular neurobiotechnology
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Increased H3K9 acetylation in PRNP promoter correlates with up-regulated PrPC expression in hippocampal samples of preclinical Alzheimer's disease

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Diagnosis at asymptomatic stages of Alzheimer's disease (AD) remains a challenge since it can only be approached at autopsy according to Braak staging (from I to VI). Achieving this would allow possible therapies to be addressed before the onset of cognitive impairment. Therefore, great efforts have been made to determine the molecular changes that occur at the onset of the disease to determine early biomarkers and intracellular signaling pathways in which to intervene therapeutically. In this sense, and according to the pathological anatomical study of human postmortem brain tissue, there is an increase of cellular prion protein (PrPC) expression in the asymptomatic stages of the disease (histopathologically characterized samples in the early stages of Braak (from I to II)). And it is known that PRNP transcriptional activity responds to tau levels, being AP-1 binding site the most functional promoter regulation region. However, to date, there are no studies on the epigenetic regulation of the PRNP promoter in the disease. egarding the role of PrPC in the disease is still a matter of debate since its neuroprotective function has already been demonstrated but its role as a receptor for toxic oligomers of beta-amyloid peptide has also been reported. It is therefore even more important to know under what circumstances and by what mechanisms their levels are regulated in the disease. Altogether, we aim to analyze epigenetic changes regulating the PRNP promoter in AD, including analysis of histone acetylation as well as cytosine methylation.

Thus, using brain human samples from diagnosed AD patients at different Braak stages (from I to VI) our results showed an increase of H3K9 acetylation associated with PRNP promoter in the first steps of the disease when compared to non-AD control samples and, a decrease at later stages, correlating with PrPC expression profile. However, non-relevant changes were found in methylated cytosines near the main transcriptional factors binding sites, suggesting that the cytosine methylation pattern remains unchanged in the disease and the increase in PRNP transcription responds only to changes in chromatin condensation.

POSTER 9 presented by:

NAME: Sheeza Mughal
GROUP: Biosensors for bioengineering
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Decoding Chronic Fatigue Syndrome and Long-Covid-19 - Mitochondrial Pathology in serum exposed 3-D *in vitro* Skeletal Muscle Tissues

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Multiple studies suggest that up to 10-15% of all patients with COVID-19 experience a range of symptoms from which some recover in 2-3 weeks, while others develop a disabling long-term sequelae picture that lasts for months or even longer after overcoming COVID-19. Long COVID-19 patients frequently report Chronic Fatigue Syndrome (CFS) or fibromyalgia, a long-term debilitating condition compromising the patient's musculoskeletal system and bringing about severe post-exertional malaise [1]. At present, there is no concrete understanding regarding disease progression, there are also no identified biomarkers and, therefore, no diagnostic tests for these patients. Our research aims to understand the pathomechanism of this condition by studying the physiological and functional impacts of patient sera on skeletal muscle function using bioengineered *in vitro* 3-D platforms. 3-D tissues were bioengineered by encapsulating human muscle satellite cells in a Matrigel-Fibrin matrix on a PDMS support. To study the effect of CFS and Long-COVID-19 sera on muscle homeostasis, mature skeletal muscle tissues were treated with patient and healthy sera. Then the tissues were characterized at structural and functional levels. On one hand, the myotube structure was analyzed by immunofluorescence techniques. On the other hand, tissues were exposed to different pulsating electric frequencies for acquiring both twitch and tetanic contractions in situ for functional characterization.

Preliminary comparative functional and structural analyses of tissues treated with patient (CFS and Long COVID-19) and healthy sera after Electric Pulse Stimulatory (EPS) training suggests a significantly weaker specific force of contraction for tissues treated with patient sera. Tissues treated with CFS patient sera showed a larger myotube diameter compared to tissues treated with healthy sera. The effective cross-sectional area of the two sample sets, however, remained the same. Quantitative Mitochondrial Morphometry indicates Stress Induced Hyperfusion. Moreover, Mitostress Test using Seahorse Analyses revealed elevated OXPHOS and non-mitochondrial respiration. Similar functional and structural implications of the two diseases on tissues point to a common mechanism of disease progression that appears to be metabolic in nature. Our key finding indicates that the disease likely progresses from a Hypermetabolic state through a gradual accumulation of ROX until it reaches atrophy. These novel findings are the first in this area to utilize static 3D bioengineered platforms to understand peripheral fatigue and ultimately demystify Chronic Fatigue Syndrome and Long-COVID-19.

POSTER 10 presented by:

NAME: Adria Noguera Monteagudo
GROUP: Biomaterials for regenerative therapies
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Development of a microfluidic system for the study of the angiogenic response in an extracellular matrix

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The process of growth of new blood vessels from existing ones is known as angiogenesis. Angiogenesis is not only a highly important biological process but also has a significant role in tissue and implant revascularization, tissue engineering applications, tumor growth and it is involved in other health conditions such as malignant disorders, inflammatory problems, ischemia, infections, and immune diseases [1]. However, recent advancements in microfluidic technology have opened the way for *in vitro* models of angiogenesis, allowing researchers to investigate its intricate mechanisms [2-3].

In this study, we have designed a microfluidic chip [4] to explore angiogenesis using isolated endothelial cells (EC). We characterized the extracellular matrix, a fibrin hydrogel, through physical and structural analysis, including rheology and fluorescent staining at micro and nano levels. Our initial cellular experiments focused on EC migration under various microenvironmental conditions demonstrate that our chip design facilitates the study of angiogenesis by enabling the formation of a VEGF angiogenic gradient.

Furthermore, we developed a mathematical model to simulate EC migration through the hydrogel, incorporating two critical microenvironmental factors: the extracellular matrix and the distribution of the chemotactic factor. The simulations aimed to capture the dynamics and morphology of angiogenesis, providing a new perspective for understanding this process and the impact of biomaterial structure.

Although further research is needed, our results demonstrate its ability to replicate functional microenvironments with precise control, which was previously only achievable in *in vivo* studies. Additionally, the developed mathematical model is expected to contribute to a better understanding of angiogenesis by enabling continuous studies over time.

POSTER 12 presented by:

NAME: Pablo Scodeller
GROUP: Choose an option
INSTITUTION: Institute of Advanced Chemistry of Catalonia (IQAC-CSIC)

Verteporfin-based Peptide-Drug conjugate modulates tumor macrophages eliciting therapeutic effect

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In triple negative breast cancer (TNBC), anti-inflammatory tumor associated macrophages (M2 TAMs) elicit metastasis, suppress the immune response, and elicit resistance to immune checkpoint blockade. Peptide-drug conjugates (PDC) based on binding peptides are promising precision delivery systems that display high tumor penetration, high selectivity, and low fabrication cost. Here, to target CD206-overexpressing M2 TAMs, we first engineered a previously identified CD206-binding peptide, mUNO, to enhance its affinity and proteolytic stability, and subsequently designed a PDC able to modulate TAM phenotype. The new peptide, TrimUNO, was designed based on the Sunflower Trypsin Inhibitor-I (SFTI-I) and studied using molecular dynamics and docking.

Computational modeling showed interaction of TrimUNO with the interlectin domain of CD206 and high conformational stability. Binding studies to recombinant CD206 using Quartz Crystal Microbalance (QCM) revealed a KD of ~0.4μM, a 15-fold higher affinity than parental mUNO. LC-MassSpec revealed a 5-fold higher half-life in breast tumor lysate of TrimUNO respect to mUNO. Homing studies in breast tumor-bearing mice showed that Fluorescein (FAM)-TrimUNO precisely targeted M2 TAMs using intravenous, intraperitoneal, and even oral administration, with no significant accumulation in control organs. We designed a simple PDC, referred to as "VetrimUNO", by coupling TrimUNO to the FDA-approved drug Verteporfin, an inhibitor of the YAP/TAZ pathway. *In vitro* studies on primary mouse and human macrophages revealed that VetrimUNO increased the expression of the class II major histocompatibility complex (MHC II), greatly stimulated the production of the inflammatory cytokines TNF-α, IL-12p40 and IL-1β and increased cytosolic YAP. In the highly metastatic 4T1.2 TNBC model, treatment with VetrimUNO slowed down the primary tumor growth and suppressed

pulmonary metastases, without inducing bodyweight loss. Flow cytometry analysis of the tumor microenvironment showed that VetrimUNO increased the MHCII+/CD206+ TAM/monocyte population by 20% and decreased the CD206+/MHCII- population by more than 10%. VetrimUNO doubled the proportion of T cells in the tumor and. In both inguinal lymph nodes, an increase in CD25+ follicular B cells was detected in the VetrimUNO group.

Our studies propose VetrimUNO as a useful PDC conjugate to modulate macrophage function in the context of solid tumors. Future combinational therapies will determine if VetrimUNO is a possible adjuvant to immune checkpoint blockade for the treatment of TNBC.

POSTER 13 presented by:

NAME: Ainoa Tejedera Villafranca
GROUP: Biosensors for bioengineering
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Mimicking Sarcolemmal Damage *in vitro*: A Contractile 3D Model of Skeletal Muscle for Drug Testing in Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is the most prevalent neuromuscular disease diagnosed in childhood. It is a progressive and wasting disease, characterized by a degeneration of skeletal and cardiac muscles caused by the lack of dystrophin protein. The absence of this crucial structural protein leads to the fragility of the sarcolemma, resulting in muscle fiber damage during contraction. Despite ongoing efforts, there is no cure available for DMD patients. One of the primary challenges is the limited efficacy of current preclinical models, which fail in the reproduction of the biological complexity of the disease. Human-based 3D cell culture methods appear as a novel approach to accelerate preclinical research, as they improve the mimicking of pathophysiological processes of the skeletal muscle. In this work, we developed a patient-derived functional 3D skeletal muscle model of DMD that reproduces the sarcolemmal damage found in the native DMD muscle. These bioengineered skeletal muscle tissues exhibit contractile functionality, as they responded to electrical pulse stimulation (EPS). Sustained contractile regimes induced the loss of myotube integrity, mirroring the pathological myotube breakdown inherent in DMD due to sarcolemmal instability. Moreover, damaged DMD tissues showed disease functional phenotypes, such as tetanic fatigue. We also evaluated the therapeutic effect of utrophin upregulator drug candidates on the functionality of the skeletal muscle tissues, thus providing deeper insight into the real impact of these treatments. Overall, our findings underscore the potential of bioengineered 3D skeletal muscle technology to advance DMD research and facilitate the development of novel therapies for DMD and related neuromuscular disorders.

POSTER 14 presented by:

NAME: Anna Vilche
GROUP: Biomaterials for neural regeneration
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Creating a Brain Culture System: Microphysiology and Hydrogel Integration

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The rising prevalence of neurological diseases poses a significant societal and mental health challenge, particularly as the population ages and life expectancy increases.1 However, the progress in studying and developing drugs for these conditions is impeded by the limited physiological representation and reproducibility of current 2D and 3D study models, hindering effective research and treatment advancements.2 Fortunately, microphysiological devices now offer the ability to simulate important aspects of central nervous system physiology in the laboratory, which is critical for understanding and studying neurodegenerative diseases among others. In this research, our goal is to create a brain culture system within a microphysiology device. This system will contain various types of neural cells embedded in a hydrogel that mimics the extracellular matrix (ECM).

The system consists of a polydimethylsiloxane (PDMS) microphysiological device adhered to a glass coverslip with a central channel and two lateral channels that transport the different substances from the 4 reservoirs. The hydrogel located in the central channel of the device, which acts as ECM, is composed of methacrylated gelatin (GelMa) as a backbone, modified with methacrylated alginate and hyaluronic acid, which allows the lowering of the material's stiffness to match the central nervous system (CNS) one. The characterization of the physicochemical properties of the hydrogel shows a highly interconnected porosity, low stiffness, high water content, and low degradability allowing a close imitation of the extracellular matrix.

The current research uses a culture system in which glial cells are seeded into the central canal simultaneously with the hydrogel, cross-linked with UV light, and neuronal cells are placed in the reservoirs for their subsequent migration into the central canal. The culture of astrocytes showed a long survival, adhesion, and growth time, conditioning

the environment for co-culture with neurons, facilitated by the neuroprotective nature of the hydrogel. This allowed the development of a homogeneously distributed neural network, promoting a neural co-culture system.

Therefore, this system is composed of a microphysiological device, and the hydrogel provides a neuroprotective character in long-term coculture, which gives this tool great potential as a neuronal model, allowing the study of different diseases and the development of treatments.

POSTER 15 presented by:

NAME: Renato Eduardo Kevyn Yanac Huertas
GROUP: Molecular and cellular neurobiotechnology
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

A microfluidic platform as a physiologically relevant myocardium model for cardiovascular diseases

Eduardo Yanac Huertas [1,3], Matilde Roquette [1], Anna Vilche Mariscal [1,5], Adrià Noguera Monteagudo [1,2], Shana Jin [1,2], Romén Rodríguez [1,4], J. A. Del Río [3], O. Castaño [1,2,6,7]
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Heart cardiovascular diseases (HCVD) are nowadaysone of the leading cause of global death. Treatment includes lifestyle changes, medication, and surgery. Regenerative therapies involving 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.

Among these diseases, some affect the myocardium, a complex and specialized cardiac tissue composed of fibroblasts, smooth muscle cells, and cardiomyocytes. The extracellular matrix (ECM) surrounding myocytes and myofibers contains collagen, elastin, fibronectin, laminin, and proteoglycans. Taking all this into account, it was proposed to synthesize a hydrogel of methacrylated gelatin (GelMa) and methacrylated hyaluronic acid (HaMa) that could replicate the properties of the ECM.

In this study, we designed a microfluidic chip, synthesized a hydrogel based on GelMa and HaMa, and cultured myocardial cells on aligned PLA fibers. The hydrogel was characterized by rheology, compression, and mass swelling. Cell alignment was analyzed by fast Fourier transform. Calcium transients of 2D and 3D myocardial cultures, infected with GCaMP, were analysed by our own software.

The results show that we have achieved a stiffness of 7.4k ± 0.96kPa, which is optimal for use as a cardiac scaffold. We achieved the first objective of cell culture by reproducing a 2D anisotropic tissue, using aligned PLA fibers by electrospinning. Then an initial 3D myocardial culture was also achieved, this model is currently being optimized to obtain the correct cell density for good growth. In the first tests, cell viability of 80.9% was obtained on the first day, and then decreased; however, in areas with high cell density on the third day, high connectivity of Ca2+ transients was observed.

Based on our results, we will extend the setup to sense this 2D and 3D cardiac tissue with gold interconnected electrodes to obtain an electrocardiogram and the impedance tissue. Improved outcomes would benefit cardiac research by offering a strong microphysiological model for testing regenerative treatments and analyzing them via calcium traces.

POSTER 16 presented by:

NAME: Karolina Zimkowska
GROUP: Molecular and cellular neurobiotechnology
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Project Outline: 3D Brain Organoid Tauopathy Model

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Neurodegenerative tauopathies are characterised by aberrant deposition of tau protein in the brain, which occurs in a stereotypical progression between anatomically connected regions corresponding to neural networks involved in memory. The currently available models lack the necessary complexity of the human brain to fully understand the underlying mechanisms of tau seeding and propagation. To address this, we present a novel *in vitro* platform utilizing cortical organoids derived from human pluripotent stem cells (hPSCs). Our study aims to unravel the complexities of tau pathology and its spread within a more physiologically relevant neural environment.

Differentiated from hPSCs, cortical organoids represent three-dimensional structures that recapitulate the organization and functionality of the human cortex. By utilising this model system, we aim to investigate the impact of pathological tau on neuronal activity in cortical organoids. Calcium imaging techniques will be employed to analyse changes in neuronal activity patterns and their correlation with tau pathology. Additionally, we will explore whether tau seeding and its propagation are dependent on neuronal activity by manipulating levels using optogenetic techniques. These investigations will elucidate the interplay between tau pathology and neuronal activity, providing valuable insights into functional consequences.

In addition to cortical organoids, we propose the utilisation of assembloids, a cutting-edge technique merging two organoids: one infected with pathogenic tau and the other serving as a control. This controlled experimental setting enables the investigation of tau propagation between distinct organoids. By analysing the interaction and communication between the pathogenic tau-infected organoid and the healthy organoid, we aim to shed light on the mechanisms involved in the intercellular transmission and spread of tau pathology. Integration of these two organoids will allow

us to observe the propagation of pathogenic tau species from the infected to the healthy organoid, providing insights into the kinetics and dynamics of tau spreading.

By addressing the limitations of existing models and incorporating advanced methodologies, our study seeks to advance our understanding of tauopathies and their pathological mechanisms. The findings from this research may pave the way for the development of new therapeutic strategies and interventions targeting tau propagation in neurodegenerative diseases.

POSTER 17 presented by:

NAME: Dolores Blanco-Almazán
GROUP: Biomedical signal processing and interpretation
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Evaluating Breathing Pattern in COPD Patients using Thoracic Bioimpedance

Dolores Blanco-Almazán [1,2,3], Willemijn Groenendaal [4], Lien Lijnen [5], Rana Önder [5], Christophe Smeets [6], David Ruttens [5,7], Francky Catthoor [8], Raimon Jané [1,2,3]
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Breathing pattern analysis is a valuable tool in understanding respiratory control in several conditions such as chronic obstructive pulmonary disease (COPD). Breathing pattern has been shown to be different in COPD patients compared to healthy controls during rest and walking. Therefore, the monitoring of breathing pattern variability is a potential source of information on respiratory control in different conditions. Previous studies have shown that thoracic bioimpedance can be used for detecting respiratory cycles and for estimating breathing pattern parameters. This study aimed to assess respiratory parameters and breathing variability using thoracic bioimpedance in COPD patients, considering the severity of the condition.

We recruited 66 COPD patients and monitored thoracic bioimpedance during the six-minute walk test (6MWT), as well as during 5 minutes before and after the test (resting and recovery phases). Patients were classified into two groups based on their spirometry test results which evaluate the severity of the airflow limitation (moderate and severe groups).

To characterize the breathing patterns, we computed common respiratory parameters, including inspiratory, expiratory, and total times, duty cycle, respiratory rate, and bioimpedance amplitude. The analysis focused on wearable bioimpedance data exclusively. Median and coefficient of variation (i.e., standard deviation over mean) values were calculated for each parameter during the three phases of the protocol, and statistical differences between the COPD severity groups were evaluated.

The results revealed significant differences between the COPD severity groups exclusively during the sitting phases, while the behavior during the 6MWT remained similar. Notably, we observed an inverse relationship between breathing pattern

variability and COPD severity. This suggests that patients with severe COPD exhibited more restricted breathing compared to those with moderate COPD. During the walking phase, both groups showed comparable behavior which may indicate a lack of relation between the regulation of breathing and COPD severity under an increase in metabolic need.

This study reinforces the potential of thoracic bioimpedance as a non-invasive tool for monitoring breathing pattern in COPD patients. Understanding the relationship between breathing patterns and disease severity could contribute to better management and personalized treatment approaches for individuals with COPD.

POSTER 18 presented by:

NAME: David Gomez-Cabeza
GROUP: Molecular imaging for precision medicine
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

CheShImP: Democratising MRSI Data Processing of Chemical Shift Images for Precision Medicine

David Gomez-Cabeza [1], James Eills [1], Marc Azagra [1], Irene Marco-Rius[1]
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The highly complex profile of multiple diseases, such as non-alcoholic fatty liver disease (NAFLD), drives researchers to seek holistic yet patient-tailored treatments to tackle these highly pressing health issues. Organ-on-a-chip systems present an attractive platform to achieve this ambitious precision medicine objective. These devices allow the modelling of patient-derived organs and tissues to understand disease metabolism and, ultimately, optimise their therapy. Magnetic resonance spectroscopic imaging permits us to track in real-time cell metabolism by tagging relevant molecules with 13C and using hyperpolarisation methods to greatly enhance the 13C MRI signal. After administration, the compound is uptaken into cells where it can undergo metabolism. We can use these non-invasive methods in MRI systems to study metabolic flux in real-time with spatial resolution of the order of millimetres thanks to chemical shift imaging (CSI), which provides a unique signature for different molecules. However, the general lack of tools to process and visualise this highly complex data deter their use amongst inexperienced groups. Here, we present CheShImP, an easy-to-use and open-source platform to handle chemical shift images, contributing to the spread of this technique. The program works for Bruker MRI systems, extracting FID and acquisition metadata files to automatically process these and provide users with grid images containing the localised spectroscopic data, optionally overlayed over localisation proton images. We used this interactive software to analyse *in vitro* the use of hyperpolarised 13C-1-pyruvate for metabolic imaging. The conversion of this molecule to different substrates (e.g., alanine or lactate) in cells or organoids cultured in microfluidic chips will allow us to study the metabolism of different stages of human diseases (e.g., NAFLD) and discover the drugs and treatments required to bring back metabolism to nominal healthy levels. Hence, this computational tool will facilitate the use of CSI for pre-clinical and clinical studies focused on the final goal of precision medicine.cells or organoids cultured in microfluidic chips will allow us to study the metabolism of different stages of human diseases (e.g., NAFLD) and discover the drugs and treatments required to bring back metabolism to nominal healthy levels. Hence, this computational tool will facilitate the use of CSI for pre-clinical and clinical studies focused on the final goal of precision medicine.

POSTER 19 presented by:

NAME: Mariano Martín
GROUP: Protein phase transitions in health and disease
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

The mutational landscape of a mammalian functional amyloid

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Amyloid aggregates, often considered exclusively pathological entities associated with neurodegenerative diseases, have been shown to instead be functional in an increasing number of biological processes. Several functional amyloids have been identified across organisms, including mammals. RIPK3 is a serine/threonine kinase involved in the regulation of programmed cell death through necroptosis. The RIP homotypic interaction motif (RHIM) in this protein plays a crucial role in driving the formation of functional amyloid fibrils which assemble into the necrosome, a platform for signal amplification and activation of downstream pathways leading to necroptosis. The necrosome facilitates the recruitment and activation of other signalling molecules, including the mixed lineage kinase domain-like protein (MLKL), leading to necroptotic cell death. Here, we combined deep mutational scanning to an aggregation reporter to quantify the effects of missense and indels mutations on the aggregation of the RHIM domain of RIPK3. The resulting genetic landscape reveals a set of four residues which are likely to constitute the core of the functional amyloid. These residues correlate with evolutionary scores, revealing that they were selected for nucleation and amyloid assembly during evolution. Furthermore, the dataset also reveals gatekeeper residues that act to prevent excessive amyloid formation. Understanding the structural and functional properties of RIPK3 amyloids is vital for the development of therapeutic strategies as targeting the assembly of these amyloids may hold promise for modulating cell death. Furthermore, unravelling the mechanisms underlying the self-assembly of RIPK3 will generate insights on how in evolution the aggregation of specific sequences has been selected for, rather than against.

POSTER 20 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 human organ, 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 extremely delicate to alterations from the outside environment, that is, the blood circulation. The brain tissues recondition endothelial cells to create a unique vasculature called the blood-brain barrier (BBB). This acts as a metabolic barrier between the central nervous system and the rest of the body.). This barrier comprises endothelial cells that tightly wrap the capillaries and applies strict control over the molecules that enter and exit the brain. In this control, the membrane proteins called receptors in the BBB play a fundamental role by binding to the molecular agents and activating the inwards/outwards transport mechanism. The present research focuses on the structure and function of a specific receptor and the low-density lipoprotein receptor-related protein 1, LRP1. This receptor comprises 4544 amino acids, around 1200 of whichh 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 [1][2] and to activate a peculiar and very efficient transport mechanism [3]. No crystal structure of LRP1 is currently available. Hence the receptor structure has been predicted with the AlphaFold2 deep learning [4]. Recently, a membrane protein closely related to it, LRP2, has been resolved [5]. 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 in proposing a new structure for LRP1 using a bioinformatic method 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 atomistic Molecular Dynamics (MD) simulations. The MD results will enable us to speculate on the structural characterization of LRP1 and the role of the calcium ions and glycans in the evolution of its flexible domains. The behaviour of the flexible domains in water agrees with the expected behaviour of the coiled conformation of LRP1 at neutral pH.

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POSTER 21 presented by:

NAME: Juan Francisco Abenza Martínez
GROUP: Integrative cell and tissue dynamics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Mechanical control of the fibroblast circadian clock via YAP/TAZ

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Cells sense and respond to the mechanical properties of their external environment. This sensing is accomplished thanks to a diverse set of biochemical pathways which impact on gene expression, subsequently affecting key cellular processes like proliferation and differentiation. Very recently, mechanics has been observed to also affect circadian rhythms, further broadening its importance in tissue homeostasis.

Our project aims to clarify the influence of mechanobiological hallmarks on the regulation of the fibroblast circadian clock. We have used NIH3T3 cells expressing Venus fluorescent protein under the promoter of the circadian gene Rev-erbα (RevVNP), confocal microscopy and customised computational analysis. Our results indicate that RevVNP expression depends on cell density. By performing gap closure experiments, we observed that basal and circadian RevVNP expression, typically low and rhythmic, is perturbed upon cell migration.

To disentangle the pathway that influences Rev-erbα transcription upon cell density changes, we used fibronectin micropatterning. Confined cells on single cell-sized areas displayed RevVNP circadian oscillations like those of confluent cells. Next, we stopped the migration of cells at low density by altering their actin dynamics with jasplakinolide and latrunculinA and observed the striking emergence of robust circadian oscillations, unlike the case of untreated single cells.

We then checked the localization of two prototypical mechanosensitive transcriptional regulators, YAP/TAZ and MRTFA, in the aforementioned compendium of conditions. We observed a strong anticorrelation of RevVNP circadian robustness and YAP/TAZ nuclear levels but not with those of MRTFA. To test if YAP/TAZ regulate the clock directly, we overexpressed dominant positive mutants of YAP/TAZ. This caused a huge impairment of the RevVNP oscillations, which demonstrates a novel role of YAP/YAZ as a circadian modulator.

Considering the role of YAP/TAZ as core mechanosensors and the metabolic importance of REV-ERB, our findings provide a fundamental link between the largely disconnected fields of chronobiology, metabolism and mechanobiology.

POSTER 22 presented by:

NAME: Ona Baguer
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

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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 (TGFβ), 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 TGFβ 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.

POSTER 23 presented by:

NAME: Nimesh Chahare
GROUP: Integrative cell and tissue dynamics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Harnessing active viscoelasticity for synthetic epithelial morphogenesis

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Epithelial sheets are active viscoelastic materials that form specialized 3D structures suited to their physiological roles, such as branched alveoli in the lungs, tubes in the kidney, and villi in the intestine. How epithelial shape arises from active viscoelasticity and luminal pressure remains poorly understood. Here we developed a microfluidic setup to engineer 3D epithelial tissues with controlled shape and pressure. Through this approach, we subject the tissues to a range of lumen pressures at different rates and probe the relation between strain and tension in different regimes. Slow pressure changes relative to the timescales of actin dynamics allow the tissue to accommodate large strain variations. However, under sudden pressure reductions, the tissue buckles and folds to store excess tissue area. This behavior is well captured by a 3D computational model that incorporates the turnover, viscoelasticity, and contractility of the actomyosin cortex. Informed by this model, we harness the active behavior of the cell cortex to pattern epithelial folds by rationally directed buckling. Our study establishes a new approach to engineering epithelial morphogenetic events.

POSTER 24 presented by:

NAME: Eleni Dalaka
GROUP: Integrative cell and tissue dynamics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Quantifying cellular forces in native tumour environments in 3D co-cultures of colorectal tumoroids and stromal cells

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Cancer is one of the leading causes of death worldwide, with metastatic cancer types presenting a high fatality rate. The tumour microenvironment (TME) has been identified as one of the main contributors of disease progression and metastasis, via biochemical signals transduction, chemotaxis and pathway activation. The past few years, however, several studies have focused on the mechanical aspect of TME and have shown that the stiffening of the extracellular matrix (ECM) at peritumoral areas drives cancer invasion and metastasis. However, the contribution of stromal contractility in cancer progression is poorly understood, while the precise quantification of mechanical forces between cancer and stromal cells remains elusive.

Here, we investigate the role of cancer-associated fibroblasts (CAFs) in the promotion or restriction of colorectal tumoroids. More precisely, we measure 3D cellular forces exerted by CAFs, and we study how these forces affect the behaviour and mechanics of colorectal tumoroids. Our model system consists of 3D co-cultures of colorectal mouse tumoroids (MTOs) and fibroblasts. We follow a new approach of preparing 3D co-cultures, by forming 3D cellular spheroids of MTOs and fibroblasts, avoiding the use of artificial matrices. The cancer-fibroblast cell ratio, the co-culture conditions and timing have also been optimized for our system. Fibroblasts have the ability of secreting extracellular matrix (ECM) proteins (e.g. fibronectin, collagen-I, tenascin etc.), thus creating a natural ECM and recapitulating fully the stroma of a native environment *in vitro*. These co-culture spheroids can be further embedded in ECM matrices, on both glass and soft substrates.

For our mechanical investigation, we use soft polyacrylamide (PAAm) fluorescent beads, a recently developed approach for 3D force measurements. When introduced in the MTO-fibroblast spheroid, the PAAm beads are randomly distributed in the co-culture spheroids, allowing for 3D, local force measurement at different areas of the TME, or tensile stresses between cancer cells and fibroblasts. As the cells in the spheroid exert forces, they deform the PAAm beads in a 3D manner. By imaging the total volume of each bead over time and knowing its mechanical properties, we can calculate the stress that experiences on its entire surface, at every given time point. Accessing many different PAAm beads in the co-cultures, we will be able to measure the mechanical activity of fibroblasts and study the 3D mechanics of native TME, for the first time.

POSTER 25 presented by:

NAME: Laura Faure
GROUP: Cellular and molecular mechanobiology
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Cell confinement in 3D leads to cell extensile forces

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From tiny sperm cells to the star-shaped neurons, how cells size and shape are link to their functions have not cease to question and amaze scientists. More recently, cell morphology has been correlated to their response to mechanical signal with cell spreading being associated with their mechanical activity. However interesting, most studies use two-dimensional (2D) systems and thus do not recapitulate to the full extent three-dimensional (3D) cell shape, especially in the case of epithelial cells, though we know the importance of mechanical homeostasis inside the epithelium and its role in wound healing.

In this work, we have developed a system of structured hydrogel that enables us to measure, in 3D, the forces exerted by a single cell of controlled morphology. With it, we report a novel phenomenon in which breast epithelial cells exert extensile forces on their environment, and not only contractile forces as previously described. Moreover, we demonstrated that the shift from contractile to extensile is correlated with a diminution in cell volume and that, though, the actin cytoskeleton plays a role in both behaviours, the myosinII is only implicated in the contractile activity. More generally, this raise the question of the importance of such a phenomenon in epithelia where the cell volume and mechanical homeostasis need to be constantly maintained through cell division and cell death.

POSTER 26 presented by:

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GROUP: Integrative cell and tissue dynamics
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Cell migration up and down fibronectin gradients

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The ability of cells to perform directed migration is essential for biological processes, such as tissue morphogenesis, immune function, and cancer invasion. Directed cell migration is often triggered by spatial gradients in the cellular environment (e.g., chemical gradients, called chemotaxis, substrate stiffness gradients, called durotaxis, or substrate-bound ligand gradients, called haptotaxis). Haptotaxis has been described *in vivo* as an important phenomenon during physiological and pathological conditions. However, the molecular and mechanical processes that drive this form of directed cell migration remain elusive. Moreover, generating accurate and reliable gradients of immobilized protein *in vitro* has been challenging and makes it harder to study haptotaxis. Here, we explore how cells sense and respond to gradients of immobilized proteins. We used a photopatterning technique to create well-controlled fibronectin gradients and we studied the migration of single mammary epithelial cells (MCF-10A). This approach allowed us to map cell migration velocity, traction forces, and actin cytoskeleton dynamics as a function of fibronectin density. We observed that cells respond to fibronectin gradients by an initial polarization towards higher protein density in the first hours of migration. Surprisingly, after this initial polarization, cells maintained their directionality even if they were submitted to a negative protein gradient. This suggests that cells adapt their polarity features to maintain the preexisting structures and organelles geometry towards low fibronectin regions until a limitation on creating new adhesions. In this work we find that one key adaptation mechanism is driven by the actin flows, specifically the increase in actin polymerization velocity at the leading edge. Besides haptotaxis, we foresee that these results will shed light on other forms of directed cell migration in which cells integrate several internal and external cues to orient themselves in physiological and pathological processes.

POSTER 27 presented by:

NAME: Miguel Gonzalez Martin
GROUP: Cellular and molecular mechanobiology
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Designing synthetic mechanosensitive molecules for the mechanical control of cellular transcription.

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Mechanotransduction is the process of transforming intracellular or extracellular mechanical signals into biochemical signals that trigger downstream cellular responses. To do so, cells have a plethora of mechanisms ranging from simple ones like the mechanical opening of ion channels in the plasma membrane, to more complex ones like the nuclear membrane stretching through the cytoskeleton, that leads into chromatin remodeling and changes in transcription. Among these, force-induced translocation of transcription factors (TFs) from the cytoplasm to the nucleus, due to alterations in the facilitated nuclear transport through nuclear pore complexes, is a straightforward mechanotransduction process with a clear impact on transcription. Taking inspiration from this mechanism, we aim to design new synthetic TFs with nucleocytoplasmic transport properties sensitive to force-driven nuclear deformations. However, synthetic TFs localize in the nucleus due to their affinity for DNA, something that we will counterbalance with the use of cytosolic retention sequences (CRSs). Creating a directed evolution screening platform based on substrate stiffness we aimed to find the best combination of mechanical properties, CRS nuclear export signals and nuclear localization signals. This platform will be the first of its kind, adapting a powerful synthetic biology tool to the field of mechanobiology and it is innovative since it screens for positive and negative phenotypes at once. Indeed, high force application to the nucleus will imply an accumulation of the transcription factor in the nucleus, enhancing transcription. The advantages of this transcription controlling system are its versatility for different cell types and simplicity. It will allow the implementation of mechano-sensitivity in transcription factors within synthetic gene circuits. Applications include creating mechano-reporters, inverting mechanical- associated phenotypes, or the creation of new set of tools for future biotechnology research in 3D structures, where forces play a major role.

POSTER 28 presented by:

NAME: Clément Hallopeau
GROUP: Integrative cell and tissue dynamics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Mechanisms of mechanical compartmentalization in intestinal organoids

Clément Hallopeau [1, 8], Gerardo Ceada [1,2, 8], Anghara Menendez [1], Sergio Palomo [3], Danijela Vignjevic [4], Pere Roca-Cusachs [1,2], Carlos Pérez-González [4], Eduard Batlle [3,5,6], Xavier Trepat [1,2,6,7]
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Monolayers of intestinal organoids recapitulate the functional compartmentalization 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 compartments (Pérez-González, Ceada et al, Nat Cell Bio, 2021). Crypt compartmentalization is attributed to a gradient in Eph/ephrin signaling, but how this gradient is linked to mechanical patterns is unknown. To address this question, we study the mechanical and functional compartmentalization in organoids derived from mice lacking EphB2 and EphB3 (EphB2-/-, EphB3-/-). Stainings and timelapses reveal an effective but weaker compartmentalization in the double knockout organoids. The size of the proliferative regions increases over time in the knockouts while remaining constant in the wild types. 3D Traction Force Microscopy shows a qualitatively similar distribution of crypt forces in the knockout, but with lower amplitude than in the wild type. Taken together, these data indicate a link between Eph/ephrin signaling and mechanical compartmentalization in intestinal organoids.

POSTER 29 presented by:

NAME: Marc Molina Jordán
GROUP: Cellular and molecular mechanobiology
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Study of the role of substrate stiffness and force transmission to the nucleus in nucleocytoplasmic transport, nuclear pore conformation, genome organization and gene expression

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The application of mechanical force to the nucleus has been recently shown to regulate important functions, including nucleocytoplasmic transport (NCT), chromatin organization, and gene expression. However, how substrate rigidity and the subsequent transmission of force to the nucleus impacts nuclear function and dynamics is still poorly understood. Here we focus on two aspects: the effect of nuclear force in NPCs and in chromatin organization and gene expression. We show how knockdown (KD) of two key NPC structural components, namely NUP155 and NUP153, decrease the accumulation of the transcriptional regulator (TR) YAP/TAZ to the nucleus as well as disrupt NCT in mammalian cells. With the aim to analyze structural changes in nuclear pores we perform super-resolution microscopy (SRM) and establish a method to image cells on substrates of different stiffness. In this set up and using U-2 osteosarcoma (U-2 OS) cells we do not observe rigidity or KD-dependent changes in NPC size. However, we suspect that these results arise from cell type or NUP-specific conditions and propose new candidates to test our new hypothesis. In addition, we use substrate stiffness to characterize via Hi-C and RNA-sequencing the force-dependent changes in chromatin state and transcription. Our results show that biomechanical manipulation using mutants to impair mechanosensitive NCT as well as substrate stiffness affects the functional organization of topologically associated domains (TADs) as well as the expression of genes linked to the cytoskeleton, focal adhesions, and the NPC.

POSTER 30 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 hPSCs

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Introduction. The mature kidney 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 taking advantage of *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 signalling pathways as key players in nephron patterning and segmentation.

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. These dynamics occurring during RV emergence and further nephron patterning are yet to be explored in the human context in real time. If these biomechanical processes are interconnected with mechanical signals remains an open question in the field. The answer to these questions may have an important impact for understanding nephron formation, and conversely, disease-related phenotypes due to mutations in genes orchestrating RV patterning and segmentation as occurs 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 (WT and CAKUT) 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 hydrogels with controlled geometries and rigidities in a 2D culture system. PDMS hydrogels of 3 kPa are generated by adapting the compositional ratio of PDMS components and are further functionalized with fibronectin. Using this system, we have started to spatiotemporally characterise early steps of nephrogenesis by immunofluorescence analysis and traction force microscopy (TFM). These analyses are conducted during RV emergence prior proximal-distal RV polarization and formation of the nephron-like segments.

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

POSTER 31 presented by:

NAME: Alice Perucca
GROUP: Integrative cell and tissue dynamics
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Micro Immune Response On-chip (MIRO): a model of tumour-stroma interface for immunotherapy testing

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Immunotherapies are offering considerable opportunities for cancer treatment, yet their benefit remains limited to 20-40% of patients. The complex interactions between cancer cells and their microenvironment play a critical role in the resistance to such treatments and call for the need of models fully recapitulating the tumour organisation to better predict drug efficiency. Here we develop the Micro Immune response On-chip (MIRO), a fully humanized platform replicating ex vivo the intratumoral spatial architecture considering the interactions between cancer cells and their immunocompetent microenvironment. We perform a comprehensive analysis of patient samples indicating that MIRO closely recapitulates the barrier composed of CAFs and CAF-secreted extracellular matrix encapsulating cancer cell nests in human tumours. We establish that stromal barriers associate with immune exclusion and protect cancer cells from antibody-dependent cellular cytotoxicity driven by targeted therapy. We demonstrate that IL2-driven immunomodulation increases immune cell velocity and spreading to overcome stromal immunosuppression and restores anti-cancer response in refractory tumours.

Collectively, our study highlights the translational value of MIRO as a powerful companion tool for precision medicine and pre-clinical drug testing.

POSTER 32 presented by:

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GROUP: Integrative cell and tissue dynamics
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Optogenetic generation of leader cells reveals a force-velocity relation for collective cell migration

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The front of migratory cellular clusters during development, wound healing and cancer invasion is typically populated with highly protrusive cells that are called leader cells. Leader cells are thought to physically pull and direct their cohort of followers, but how leaders and followers are mechanically organized to migrate collectively remains controversial. One possibility is that the autonomous local action of a leader cell is sufficient to drive migration of the group. Yet another possibility is that a global mechanical organization is required for the group to move cohesively. Here we show that the effectiveness of leader-follower organization is proportional to the asymmetry of mechanical stress within the cellular cluster. By combining hydrogel micropatterning and optogenetic activation of Rac1, we locally generate highly protrusive leaders at the edge of minimal cell groups. We find that the induced leader can robustly drag one follower but is generally unable to direct larger groups. By measuring traction forces and stress propagation in groups of increasing size, we establish a quantitative relationship between group velocity and the asymmetry of the traction and stress profiles. We propose a model of the motile cluster as an active polar fluid that explains this force-velocity relationship in terms of asymmetries in the distribution of active tractions. Our results challenge the notion of autonomous leader-cells by showing that collective cell migration requires a global organization of mechanical stresses within the cluster.

POSTER 33 presented by:

NAME: Ignacio Viciano
GROUP: Cellular and molecular mechanobiology
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Development of small molecules inhibitors of mechanotransduction as potential pancreatic cancer therapy

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Most solid tumors show an increase in tissue stifness, which drives tumor progression. Our lab has demonstrated that tissue stiffness triggers unfolding of a target protein and induces an interaction with its binding partner. Under a regime of forces, both intracellular and extracellular, Talin protein unfolds thus exposing several cryptic binding sites to the Vinculin protein. The binding between these proteins leads to the adhesion growth and YAP nuclear translocation. It is well known that YAP signaling pathway is key in many tumor processes, mainly in Pancreatic cancer.

In the present study we have working in the discovery of small molecules capable of blocking or inhibit the interaction between the proteins Talin and Vinculin. Our approach is based on the hypothesis that a small molecule can bind strongly to a specific key region of the Talin rod domain, thus hindering its unfolding, ultimately preventing the exposure of the cryptic binding sites.

To do this, we have conducted an computer-aided drug design project (CADD), that has helped us to obtain a hit molecule, as demonstrated by our cell-based assays. Currently, we are optimizing this lit with the help of medicinal chemists to obtain a lead molecule, which could become a potential pancreatic cancer therapy.

POSTER 34 presented by:

NAME: Srivatsava Viswanadha
GROUP: Cellular and molecular mechanobiology
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Characterizing the role of mechanotransduction in mouse embryonic stem cells

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Mouse Embryonic Stem Cells (mESCs) possess ground/naïve state pluripotency when grown in defined N2B27 media supplemented with two inhibitors (2i), for Erk and GSK3β [1,2]. Upon 2i removal, mESCs exit naïve state, become functionally mature and acquire differentiation competence. On the mechanical front, ground state exit is initiated by the integrin mediated mechano-sensing of extra cellular matrix (ECM) [3,4]. Although, Laminin was found to be the pivotal ECM ligand for pluripotency dissolution [3], the accompanying down-stream mechano-responses, their spatio-temporal evolution and, their regulatory role in mESC maturation are unknown. In this work, we combine functional characterization and live cell imaging to unravel the role of mESCs-ECM interactions during pluripotency dissolution. We employed a fluorescent mESC line to monitor naïve state exit in real time, in a laminin-rich ECM environment. During naïve state exit, we observe growing cell-ECM interaction, marked by a progressive increase in traction forces, lengths of focal adhesions, and basal actin reorganization. Moreover, in the later stages of mESC maturation, the edges of colonies displayed higher tractions and nuclear YAP with flattened nuclear morphology, traits of mechanical integration of the integrin-cytoskeleton-nuclear axis. Such sptially distinct expression pattern was observed for the general pluripotency factor Nanog and the pluripoency priming factor Otx2, and naive state specifier Rex1-GFP. Among the nuclear morphometrics, aspect ratio, serving as a proxy for force application to nucleus, was found to be the most reliable predictor of transcription factor expression. Attenuating the functionality of said axis by contractility inhibition or by targeting LINC complex to abrogate nuclear – cytoskeleton coupling altered naïve state exit kinetics. Moreover, in accordance with existing knowledge, the cells displaying traits of navie state exit were characterized by cell spreading and actin reorganization, and was analogus to tissue wetting which is determined by competition between cell-cell and cell-matrix adheions. Finally, when the balance was tilted in favour of fortifying cell-cell adhesion, naive state exit was significantly delayed. Therefore, through this work we present a mechanical logic involving molecular details for naive pluripotency exit.

POSTER 35 presented by:

NAME: Tom White
GROUP: Nanobioengineering
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Characterisation of Extracellular Matrix Models of Collagen VI-Related Congenital Muscular Dystrophies at the Nanoscale

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Collagen VI-related congenital muscular dystrophies (COL6RDs) represent a clinical-spectrum of neuromuscular conditions. These arise from deficient or dysfunctional microfibrillar collagen VI (COL6) in the extracellular matrix (ECM) of muscle and other connective tissues due to autosomal mutations in any of the three major COL6 genes. The disorders can be classified into different phenotypes according to the severity of clinical hallmarks describing motor and pulmonary function, ranging from the severe Ullrich congenital muscular dystrophy (UCMD) through phenotypes of intermediate severity to the milder Bethlem myopathy (BM). How genetic variation influences the ECM structure as well as the main mechanisms leading to the disease associated symptoms remains unclear.[1] To investigate these questions, previous work in our group led to development of personalised pre-clinical models of COL6-RDs with cell-derived matrices (CDMs) technology. These models allowed us to directly observe the fibrillar organisation of the ECM in samples derived from patients and compare features amongst different phenotypes.[2] Here, we focus on further characterizing the micromechanical properties of these models using atomic force microscopy-based force spectroscopy (AFM-FS). Young's elastic modulus values are calculated by fitting the Hertz model to force-displacement graphs acquired with a colloidal probe. Our results show a variation in ECM modulus value with different patient phenotypes, thus presenting a novel COL6-RD marker that could contribute to the label-free, early diagnosis of the disease in the form of ECM stiffness. In addition to providing new insights into the biomechanics underlying patient symptoms, we demonstrate how performing AFM-FS studies on the patient-derived cell matrices can be further applied in evaluating Nuclei Acid Therapeutics (NAT) as a treatment for different COL6-RDs.

POSTER 36 presented by:

NAME: Joana Admella Pedrico
GROUP: Bacterial infections: antimicrobial therapies
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Investigating Bacterial Infections in Galleria mellonella Larvae: Insights into Pathogen Dissemination and Behavior

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The insect Galleria mellonella is an alternative animal model widely used for studying bacterial infections. It presents a wide range of advantages, including its low cost, easy maintenance and lack of ethical constraints. Among other features, their innate immune system is very similar to that of mammals. In this study, we dissected several larvae infected with important human pathogens: Mycobacterium abscessus, Staphylococcus aureus and *Pseudomonas aeruginosa*. By observing the fat body, gut, trachea, and hemolymph under the microscope, we were able to describe where bacteria tend to disseminate. We also quantified the number of bacteria in the hemolymph throughout the infection course and found significant differences between the different pathogens. With this work, we aimed to better understand the behavior and dissemination of bacteria in the infected larvae.

POSTER 37 presented by:

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Targeting macrophage polarization states for precision immunotherapy

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Macrophages are crucial immune system components, safeguarding our tissues from external threats such as injuries, toxins, and infections [1]. When faced with an insult, resident macrophages initiate the inflammatory process, transitioning from a resting state (M0) to an activated state and changing their effector function into a pro-inflammatory (or M1) and anti-inflammatory (or M2) phenotype [2]. This dynamic activation of macrophages plays a pivotal role in disease progression and can lead to unresolved inflammation if impaired. To address this, macrophage-targeting nanomedicines have emerged as a revolutionary approach for treating a wide range of human diseases, including infections, chronic inflammatory disorders, neurodegenerative diseases, and cancer. Traditionally, targeted strategies have relied on high-affinity ligands like antibodies. However, these interactions can lead to indiscriminate targeting of any cell expressing the corresponding receptor, resulting in a loss of selectivity. One strategy to overcome such a challenge involves employing low-affinity ligands within a multivalent scaffold, thereby achieving super-selectivity [3]. This approach relies on the collective effect of individual affinities, ensuring that associations only occur when receptors are expressed at specific densities, effectively targeting cells expressing the desired receptor while minimizing non-specific interactions. We propose using engineered polymer-based self-assembled nanoparticles (polymersomes) where multiple ligands are expressed alongside polymers that prevent non-specific interactions and act as steric modulators [4]. *In vitro* experiments show that nanoparticle binding to the cell surface is non-linear, dependent on the number of ligands present. This behavior allows for identifying an optimal ligand density, creating on-off association profiles that enable precise targeting of specific macrophage phenotypes. Through this approach, we can achieve phenotypic targeting of macrophages while enhancing selectivity and therapeutic efficacy.

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POSTER 38 presented by:

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GROUP: Bacterial infections: antimicrobial therapies
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Insights on the regulation and transcription pattern of the Ribonucleotide Reductases of *Burkholderia cenocepacia* in infection-like conditions.

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Burkholderia cenocepacia is a ubiquitous multidrug-resistant Gram-negative bacteria often isolated from the lungs of cystic fibrosis patients. It is associated with reduced patient survival and an accelerated loss of lung function. Moreover, it is responsible for a fatal form of infection, *Cepacia* syndrome, an acute lung failure that often leads to necrotizing pneumonia. However, as it is not isolated as often as other lung pathogens despite its deadliness, it has been understudied in all senses, from its infection process to its most essential enzymes. Ribonucleotide Reductase (RNR) is the only enzyme responsible for reducing ribonucleotides (NDPs or NTPs) to their corresponding deoxyribonucleotides (dNDPs or dNTPs). Thus, it is in charge of the de novo synthesis of the building blocks for DNA synthesis and repair, so it is essential to sustain life. In this study, three genes belonging to two different RNR classes were found through a bioinformatic search in the existing genomic annotations of *Burkholderia cenocepacia*. Promoter and transcription factor binding site predictions were made in silico for the three genes that were found to correspond to RNRs. Also, transcriptional fusion assays with GFP were performed to describe the RNR gene transcription pattern in planktonic cultures at different growth stages and environmental conditions, as well as in static and dynamic biofilms. In addition, the effects of vitamin B12 and hydroxyurea were tested in different oxygen availability conditions to understand the RNR transcription pattern and the role of each RNR on the viability of *Burkholderia cenocepacia*. In those same oxygen conditions, RT-qPCRs of the RNR genes were performed to elucidate their transcription pattern precisely. Last, a protein homology comparison was conducted among the RNR amino acid sequence from *Burkholderia cenocepacia* and other relevant bacterial species, and a phylogenetic tree was built for the three RNR proteins. This study sets the grounds for *Burkholderia cenocepacia* RNR transcription behavior in infection-like environment studies and opens the door to considering RNRs as a potential antimicrobial target for this multidrug-resistant bacteria.

Acknowledgements

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POSTER 39 presented by:

NAME: Marc Alorda Carreras
GROUP: Signal and information processing for sensing systems
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Processing of serological microarray data for COVID-19.

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The novel SARS-CoV-2 was described as the causative agent of COVID-19 disease. A wide symptomatology arise as consequence of infection and the immune response has been directly associated with differences in clinical progression. Current serological assays measure the presence of immunoglobulins (IgG or IgM) based on viral proteins lacking additional immune characterization.

Procedure: A cohort of 755 serum samples (pre-pandemic, PCR-negative, and PCR-positive) were analyzed to validate the immunoarray identifying IgG profiles among 35 epitopes of SARS-Cov-2 protein (nucleocapsid and spike) for each patient. In order to evaluate the performance of the array in classifying patients according to the presence or absence of the infection and the severity of the disease, we have applied two types of data analysis techniques: univariate, where correlation of peptides is studied and ROC and AUC values were calculated individually, and multivariate, where classification models were trained using machine learning algorithms. Also, selection of the most predictive or discriminatory features in the data to classify samples has been calculated.

Results: The serological array was validated in clinical samples, offering 98% specificity and 91% sensibility (2% SD) over RT-PCR+ patients. Immunodominant peptides belonging to S2 subunit and N protein were identified. Computational analysis was utilized to correlate patients' serological signatures with clinical progression. Significant predictive values were obtained against lethal outcomes with 81% accuracy (exitus), ICU admissions with 70% accuracy, and 66% accuracy over hospitalization in comparison with asymptomatic or mild disease patients. Peptides with the highest predictive performance in all classifications have been identified.

Keywords: Microarray, Serological signature, Univariate analysis, Multivariate analysis, Feature selection, Machine learning.

POSTER 40 presented by:

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GROUP: Bacterial infections: antimicrobial therapies
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Died or not dyed: Assessment of viability and vitality dyes on planktonic cells and biofilms from C. parapsilosis

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Candida parapsilosis has emerged as a causative agent of invasive Candida infections, demanding effective treatment strategies. Cell viability/vitality assays are crucial for evaluating treatment efficacy, with stain-based methods offering speed and objectivity. However, there is a lack of consensus and established guidelines for their application in yeast research. This study aimed to assess the performance of four commonly used viability and vitality dyes on C. parapsilosis planktonic cells and biofilms.

Viability assessment of overnight cultures and biofilms of C. parapsilosis (treated and not-treated with amphotericin B) was conducted using Syto-9 (S9), Thiazole Orange (TO) and Propidium Iodide (PI). Metabolic activity was determined using Fluorescein diacetate (FDA) and FUN-1. Calcofluor white (CW) was employed as a control for cell visualization. Confocal microscopy and Image J-COMSTAT2 quantification were used to calculate the percentage of viability/vitality for each dye. Crystal Violet assay (CV) and Presto Blue assay (PB) were performed as standard techniques for evaluating treatment efficacy.

Heterogeneity in fluorescence intensity and permeability issues were observed with S9, TO and FDA, influenced by cell morphology. Viability and vitality percentage varied depending on the dye used, with FUN-1 showing comparable results to the PB assay. PI and FUN-1 showed good performance for C. parapsilosis staining.

These findings emphasize the importance of evaluating dye permeability in specific yeast species and growth modes and recommend conducting microscopic evaluations using control dyes for cell visualization. Inadequate dye selection could lead to misinterpretation of treatment efficacy.

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POSTER 41 presented by:

NAME: Yunuen Avalos
GROUP: Nanomalaria
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Application of aptamers targeting ESCRT-III proteins as a potential antiplasmodial tool

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Extracellular vesicles (EVs) mediate the transfer of molecules between cells and play diverse roles in host–pathogen interactions. In the case of malaria, it has been observed a significant increase in EV population upon *Plasmodium falciparum* invasion into red blood cells (RBCs) [1]. Over the past years, it has been demonstrated that the Plasmodium parasite utilizes its own protein network to release EVs [2, 3]. One of the key players for EV biogenesis in higher eukaryotes is the Endosomal Sorting Complex Required for Transport (ESCRT) machinery, which comprises ESCRT-I, -II, -III, ALIX/Bro1 and VPS4 sub-complexes that are assembled in the membranes in a sequential manner [4]. However, the ESCRT machinery in *P. falciparum* operates through a non-canonical and minimal pathway in which PfBro1 activates PfVps32 and PfVps60, both ESCRT-III members, triggering EV biogenesis [3]. In previous assays, we have demonstrated the importance of this machinery through the disruption of the Pfvps60 gene by CRISPR/Cas9 edition, which led to a significant reduction in the number of produced EVs [3]. Moreover, by using antibodies directed against PfVps32, a significant reduction in the parasite viability was observed in growth inhibition assays. Therefore, we have explored the use of DNA aptamers (also known as chemical antibodies) as a strategy to target the ESCRT-III proteins of *P. falciparum*. Aptamers offer a wide range of advantages over antibodies including high stability, rapid, inexpensive and animal-free production, ease of customized modification, small size, and potential use for specific targeted therapies [5]. We have successfully isolated aptamer pools enriched in oligonucleotides directed against the PfVps4 and PfVps32 proteins, which specifically recognized Plasmodium-infected RBCs. Furthermore, purified single DNA aptamer sequences have been tested for their antiplasmodial activity, showing a mild effect against the parasite's viability and specific binding towards Plasmodium late stages.

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POSTER 42 presented by:

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Designing hybrid polymersomes for thermoplasmonics and targeted photothermal therapy

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Polymersomes – vesicles self-assembled from amphiphilic block copolymers – are promising nanocarriers for the precise intracellular delivery of therapeutic agents and diagnostic probes. [1] Integrating inorganic light-absorbing materials, such as gold, in their membrane is a stepping stone to enable their use for photothermal therapy. Yet, it is unclear whether such hybrid polymersomes can be produced with efficient thermoplasmonic properties without altering the morphology, stability and cell-targeting functionality of polymersomes in biological environments. Here we show that stable hybrid polymersomes, produced by the in-situ synthesis of ultrasmall (~ 2 nm) gold nanoparticles in the membranes of poly[(2-methacryloyl) ethyl phosphorylcholine]-b-poly[2-(diisopropylamino)ethyl methacrylate] (PMPC–PDPA) polymersomes, can be synthesized with excellent morphological control to generate a strong thermoplasmonic response. The collective absorption of individual gold nanoparticles enables us to obtain temperature increases in the order of 10 K in dilute suspensions of hybrid polymersomes upon laser illumination at modest power densities (< 0.14 mW/μm^2).

To rationalize our observations and predict the thermoplasmonic response of these hybrid polymersomes, we develop a theoretical framework based on a Mie theory optical model combined with convective and conductive thermal transport. We then proceed to demonstrate in-vitro the applicability of our formulations in photothermal therapy by reporting the enhanced death rate of brain cancer cells treated with hybrid polymersomes under exposure to a low intensity scanning laser. We attribute this enhancement to the intracellular accumulation of plasmonically active gold nanoparticles, for which we provide experimental evidence. In this work, the uptake is mediated by the interaction between the PMPC blocks in the polymersome brush and the SR-B1, CD36 and CD81 receptors expressed on the cell surface. [2] We envision that the nanotechnological platform here developed could be translated to other ligand-receptor combinations by engineering the polymersome surface,

thus allowing the selective targeting of specific cell phenotypes and promoting the precise delivery of effective photothermal agents to tissues and organs considered beyond the reach of traditional therapeutics.

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POSTER 43 presented by:

NAME: Marco Basile
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

OOn the Amyloid-β transcytosis across the blood-brain barrierr

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The blood-brain barrier (BBB) plays a crucial role in regulating the transport of misfolded proteins to and from the Central Nervous System (CNS). Two receptors, the low-density lipoprotein receptor-related protein 1 (LRP1) and the low-density lipoprotein receptor-related protein 8 (LRP8), are involved in this process. Research suggests that LRP1 primarily transfers amyloid-β (Aβ) across the BBB, while more studies are needed for LRP8. To encourage this process, we have developed functionalized polymeric nanoparticles that mimic the *in vivo* process by having multiple ligand-receptor affinities. To validate our theory, we have evaluated the impact of these nanoparticles on gene and protein expression and conducted a thorough analysis of our *in vitro* model of the BBB. These investigations are an essential step in determining how polymeric nanoparticles can enhance the clearance of Aβ from the CNS.

POSTER 44 presented by:

NAME: Núria Blanco-Cabra
GROUP: Bacterial infections: antimicrobial therapies
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Disturbing bacterial morphogenesis by antibiotic action

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ADEP, a new class of antibiotic acyldepsipeptides, is produced by Streptomyces hawaiiensis. This naturally occurring compound exhibits potent antibacterial activity by deregulating the bacterial caseinolytic protease ClpP. The cell division protein FtsZ emerged to be especially prone to degradation by ADEP-activated ClpP at low ADEP concentrations, thereby causing an inhibition of cell division in Gram-positive bacteria, eventually leading to cell death. This work aims to investigate the impact of ADEP on streptomycetes, a genus of antibiotic-producing bacteria that undergoes a multicellular life cycle, wherein FtsZ is not essential for their viability. By elucidating the effects of ADEP in Streptomyces, we hope to gain valuable insights into the mechanisms of action and production of ADEP antibiotics.

POSTER 45 presented by:

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Investigating chemotaxis in a minimum cell model

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Chemotaxis, the movement of cells or objects in response to concentration gradients, is a widely observed phenomenon in nature, including bacterial and neutrophil dislocation. Synthetic systems based on active matter have been developed to replicate such transport behaviour. To achieve chemotaxis in both natural and synthetic systems, being out of equilibrium and having symmetry breakage are essential. In simple models, such as microparticles coated asymmetrically with a catalyst, the coating's asymmetry creates a heterogeneous distribution of species on its surface, inducing a slip velocity that results in a drift aligned with the external concentration gradient. We have showed previously that if an enzyme is encapsulated inside a vesicle, the asymmetry can be introduced by having a heterogeneous membrane, made of different copolymers with different permeabilities. In this study, we propose a model that includes the essential elements for chemotaxis, serving as a simplified representation of natural vesicles. In synaptic vesicles, for example, transmembrane proteins are present in few units, being naturally asymmetrically disposed on the membrane. Similarly, our model consists in the encapsulation of enzymes in lipid vesicles with pore proteins regulating substrate/product transport. Tracking these liposomes in a substrate gradient revealed their chemotactic behaviour, with drift velocity controlled by the number of pores on the membrane. As the tracking was conducted in a microfluidic channel, additional effects like diffusioosmophoresis were also observed. This study encourages the investigation of the potential role of chemotaxis in natural vesicle transport.

POSTER 46 presented by:

NAME: Margarita Bulatova
GROUP: Molecular imaging for precision medicine
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Understanding the Influence of Host-Guest Interaction on Fumarate Hyperpolarization

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Hyperpolarization techniques overcome the low sensitivity and contrast limit problems of NMR and MRI but suffer from the short lifetime of the hyper-intense signals of the substrates. In the case of *in vivo* studies, one of the major factors contributing to this is the interaction with the physiological environment. This study explores the potential of prolonging the hyperpolarization lifetime by forming inclusion complexes between the target metabolite molecule (fumarate) and cyclodextrins (CDs), effectively protecting them from the environment within the macrocyclic cavity.

We present a proof-of-concept study of the influence of the host molecule β-CD on the hyperpolarization of fumarate as the guest substrate. Host-guest interaction studies were conducted using conventional NMR techniques, including 1H, 13C, and DOSY, followed by hyperpolarized NMR and MRI analysis. In D2O, the results clearly demonstrate the inclusion of fumarate within the CD cavity. The supramolecular interaction has a noticeable effect on the relaxation time T1 values of fumarate's hydrogen atoms, with a decrease attributed to the interaction between fumarate and CD's hydrogen atoms, consistent with literature values. The next step involves introducing H2O and radical species to evaluate the protective properties of the supramolecular complex on hyperpolarized fumarate. This research enhances our understanding of the influence of host-guest chemistry on hyperpolarized substrates and sheds light on potential applications in MRI for monitoring metabolic processes.

POSTER 47 presented by:

NAME: Núria Camarero Palao
GROUP: Nanoprobes and nanoswitches
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Phototrexate, a photoswitchable antimetabolite for targeted photoactivated chemotherapy and psoriasis.

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Antifolates are structural analogs of folates, essential one-carbon donors in the synthesis of DNA in mammalian cells, and they work as inhibitors of key enzymes in folate metabolism, such as dihydrofolate reductase and thymidylate synthetase. Methotrexate (MTX) was one of the first agents of this class and is still extensively used in the treatment of a variety of tumors, including acute lymphocytic leukemia, breast cancer, osteosarcoma, primary central nervous system lymphoma, and head and neck cancer. Above all, it is also commonly used in certain autoimmune diseases, such as rheumatoid arthritis or psoriasis.1 However, the clinical efficacy of MTX is often limited and compromised by toxic dose-related side effects, which lead to morbidity, interruption of the treatment, and occasional mortality. A promising approach to tackle this problem is to activate the drug exclusively at its desired place of action, thus avoiding its cytotoxic effect on healthy tissues. Light is a powerful tool in this respect as it can be delivered with high precision regarding space, time, intensity, and wavelength.

We have developed Phototrexate, the first photoswitchable antifolate, by incorporation of a photochromic unit into the structure of MTX. Phototrexate was designed to be constitutively inactive in its thermodynamically stable configuration (E isomer), while it can be activated with light (Z isomer) to locally provide the pharmacological effects of the parent drug, as confirmed in our earlier experiments *in vitro* and in zebrafish larvae.2

Additionally, we have conducted preclinical studies *in vitro* and *in vivo*, to identify and assess the potential efficacy, safety, and toxicity problems associated with PHX for both isomers. All current results indicate that Phototrexate is a drug candidate with high potential for development as an innovative light-regulated antifolate for cancer and psoriasis.

POSTER 48 presented by:

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GROUP: Bacterial infections: antimicrobial therapies
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Role of microbiota on immunotherapy outcome in a murine model of non-muscle invasive bladder cancer

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Every year, approximately 600.000 people are diagnosed with bladder cancer, and more than 200.000 people die from this disease, making it the 10th most common cancer type worldwide. High-risk non-muscle invasive bladder cancer (NMIBC) which accounts for 75% of diagnoses, are commonly treated with surgical resection of the tumor followed by weekly intravesical instillations of Mycobacterium bovis Bacillus Calmette-Guérin (BCG) immunotherapy. Despite its proven efficacy, BCG treatment fails in approximately 25–45% of patients and can cause local and disseminated infections, making alternative treatments needed. Our previous studies have demonstrated that Mycobacterium brumae, a non-pathogenic environmental mycobacterium, achieves antitumoral effects comparable to BCG, making it a potential alternative for an effective and safe treatment.

The urinary bladder, previously considered sterile, has been found to contain a microbiota described mainly using 16S rRNA sequencing techniques, which has revolutionized the field of urology research. The presence of microbiota in the bladder opens up a range of possible implications on bladder cancer tumor progression, response mycobacteria immunotherapy, and its potential application as a noninvasive predictive treatment response biomarker. Some studies have observed differences in urinary microbiota between healthy individuals and bladder cancer patients, although the results show disparity, preventing the establishment of a clear differentiated profile and further studies are needed. Understanding and characterizing the recently described bladder microbiota, as well as the implications of the intestinal microbiota on bladder cancer, is essential for the development of predictive and personalized strategies to achieve more effective treatment. In this study, the bladder and stool microbiota from a murine bladder cancer orthotopic model are characterized for the first time. Comparisons between healthy mice and mycobacteria-treated and untreated tumor-bearing mice are presented, providing a comprehensive view of the changes in the gut and urinary microbiota under each condition.

POSTER 49 presented by:

NAME: Víctor Campo Pérez
GROUP: Bacterial infections: antimicrobial therapies
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An easy, high-throughput microtiter plate screening assay to quantify and differentiate species in dual-species biofilms

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Pathogenic bacteria form biofilms during infection, and polymicrobial biofilms are the most frequent manifestation. Biofilm attachment, maturation and/or antibiotic sensitivity are mainly evaluated with microtiter plate assays in which bacteria are stained to enable the quantification of biomass by optical absorbance or fluorescence emission. However, using these methods to distinguish different species in dual-species or polymicrobial biofilms is currently impossible. Colony-forming unit counts from homogenized dual-species biofilms on selective agar medium allow species differentiation but are time-consuming for a high-throughput screening. Thus, reliable, feasible and fast methods are urgently needed to study the behavior of polymicrobial and dual-species communities. This study shows that *Pseudomonas aeruginosa* and *Burkholderia cenocepacia* strains expressing specific fluorescent or bioluminescent proteins permit the more efficient study of dual-species biofilms compared to other methods that rely on measuring total biomass. Combining fluorescence and bioluminescence measurements allows independent analysis of the different microbial species within the biofilm, indicating the degree of presence of each one over time during a dual-species biofilm growth. The quantitative strategies developed in this work are reproducible and recommended for dual-species biofilm studies with high-throughput microtiter plate approaches using strains that can constitutively express fluorescent or bioluminescent proteins.

POSTER 50 presented by:

NAME: Palash Chandravanshi
GROUP: Biomaterials for neural regeneration
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Harnessing Regenerative Potential for Spinal Cord Injury Treatment with Age-Specific Decellularized Extracellular Matrix Bioinks

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Traumatic spinal cord injury is a devastating condition affecting over 500,000 individuals annually, leading to motor function decline and severe consequences like paralysis. Spinal cord trauma induces cavitation, glial scars, and adverse alterations in the extracellular matrix (ECM) at the site of injury. Our preliminary findings reveal significant changes in functional and and regenerative potential in the ECM derived from young and old mice. Injecting decellularized ECM from young mice into a fully transected mouse model resulted in higher regenerative potential of the ECM from young mice, evidenced by increased axonal growth, reduced glial scarring, and functional recovery in mice. Hence, in our ongoing study, we focus on the development and characterization of age-specific dECM bioinks using different bioprinting techniques to treat acute and chronic spinal cord lesions in a mouse model.

POSTER 51 presented by:

NAME: Shuqin Chen
GROUP: Smart nano-bio-devices
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Swarming Behavior of Enzymatic Nanomotors: Experiments and Simulations

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Single enzymatic nanomotors acquire kinetic energy through the catalysis of chemical fuels. When a group of self-propelled nanomotors gathers in a fuel-rich environment, they self-organize into ordered groups, exhibiting intriguing swarming behaviors akin to natural phenomena like the self-organization observed in bacterial colonies, bioconvection of microbial suspensions, and the coordinated movements of fish, ants, and birds. This swarming behavior affords numerous advantages as compared with individual nanomotors, including expanded coverage and prolonged propulsion duration, thus holding great promise for future applications. However, the mechanisms underlying the swarming behavior have yet to be fully elucidated. Therefore, our study aims to investigate the formation of enzymatic swarms using a combination of experimental analysis and simulations. We examine various factors that impact the movement of nanomotor swarms, such as particle concentration, fuel concentration, and fuel viscosity. By investigating these variables, we aim to gain a deeper understanding of swarming dynamics. Additionally, to further verify our assumed mechanisms, we predict collective chemotaxis behavior through simulations, which aligns with the experimental findings.

POSTER 52 presented by:

NAME: Claudia Codano
GROUP: Molecular bionics
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Design of phenotypic anti-inflammatory nanomedicines

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In recent decades, biodegradable synthetic polymers have been largely studied for many biomedical applications. Among these, poly(propylene fumarate) (PPF) has been investigated for its wide versatility of usage, from bone tissue engineering to drug delivery and regenerative medicine. PPF is a linear polyester characterised by biocompatibility and biodegradability. Upon hydrolysis of its ester linkages, the polymeric backbone releases propylene glycol and fumarate as degradation products in the cell milieu. The challenge presented herein is to prove if such fumarate metabolites could mimic the anti-inflammatory properties of dimethyl fumarate (DMF), a drug already approved for the treatment of autoimmune disorders such as multiple sclerosis and psoriasis. We designed a poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC)-PPF copolymer that combines the anti-inflammatory features of fumarate derivatives with the superselective targeting properties of PMPC towards macrophages and dendritic cells, the most important actors in managing inflammation1. The self-assembling behaviour of PMPC-PPF copolymer into a supramolecular multivalent scaffold transposes the anti-inflammatory properties of DMF to the nanoscale, making the nanoparticle the nanodrug itself and physically driving its activity through phenotypic targeting.

PPF was synthesised by step-growth polymerisation of its diester intermediate, bis(hydroxypropyl) fumarate, to accomplish this goal in a 2-step synthesis. PPF was either functionalised with 4-cyanopentanoic acid dithiobenzoate (CPADB) for reversible addition-fragmentation chain-transfer (RAFT) polymerisation or with 2-Bromo-isobutyl-bromide for atom transfer radical polymerisation (ATRP) of PMPC monomer, monitoring the polymerisation to achieve the desired length of PMPC chains. Each polymer product was analysed by gel permeation chromatography (GPC) and nuclear magnetic resonance (NMR) to ensure its quality and conversion grade. After the copolymer characterisation, PMPC-PPF micelles were obtained by solvent-switch and characterised by Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM) to evaluate, respectively, their hydrodynamic diameter and morphology, aiming for a homogeneous population. PMPC-PPF micelles were then tested on differentiated monocytes to evaluate their cytotoxicity and anti-inflammatory properties. Preliminary studies revealed a reduction in the expression of main pro-inflammatory cytokines both in pro-inflammatory and undifferentiated phenotypes, paving the way for further analysis to confirm the potentiality of this PPF-based copolymer.

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POSTER 53 presented by:

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Nanogel-based nanomotors navigating in viscous media

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In recent years, extensive efforts have been made to effectively transport drugs to precise targets within the human body. However, one significant challenge encountered is the entrapment of the drug within highly viscous media found in the entry routes of our bodies. For instance, one prominent difficulty arises when delivering therapeutic agents into the joints, specifically in the presence of synovial fluid (SF) – a protective layer composed of highly viscous hyaluronic acid. In order to tackle this issue, scientists have devised a solution by encapsulating the drug within nanoparticles (NPs). Their primary focus has been on surface modifications of these NPs, aiming to reduce their interactions with biological matrices and effectively transform them into carriers for drug delivery.

In recent times, novel classes of self-propelled NPs, known as nanorobots, have emerged. These nanorobots possess the remarkable ability to propel themselves, enabling them to navigate through viscous media more swiftly compared to previous generations of materials. This propulsion capacity lets them enter and exit such media with greater efficiency. Indeed, some studies have explored the use of magnetically guided nanorobots to navigate through the SF.[1] Additionally, Janus silica nanorods have been developed, which utilize urease and hyaluronidase enzymes to disrupt HA and enable the nanorods to self-propel within the extracellular matrix.[2] This propulsion mechanism relies on the catalytic activity of urease, requiring high concentrations of urea. In this study, we introduce an innovative approach utilizing biocompatible organic gel-based nanorobots. 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)cystine. To enable self-propulsion, the chassis has undergone enzymatic modification, leveraging its catalytic activity. This novel gel-based nanobot 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 will load macromolecules into the nanorobots, and control their release upon pH and temperature. By conducting experiments in both *In Vitro* and Ex Vivo biological environments, we will thoroughly investigate the advantages and potential benefits of this approach.

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POSTER 54 presented by:

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Effect of swarming on the delivery efficiency of pDNA-loaded layer-by-layer PLGA nanomotors

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The promise of drug delivery systems is to deliver nucleic acids (i.e., pDNA) selectively to the target tissues and cells with increased efficacy while reducing side effects. However, there are still remaining challenges, linked to the different physiological barriers that need to be overcome,[1] Nanoparticles with autonomous motion (nanomotors), have been proposed as the next generation of nanomedicines.[2] Among them, enzyme-powered motors are at the forefront, since they can utilize physiologically relevant fuels to power motion under *in vivo* conditions.[3] Recent studies have demonstrated that urease presents high catalytic rates that confer to urease-functionalized motors higher self-propelling capabilities compared to other enzymes.[4] Moreover, their collective behavior (what it is commonly referred as swarming behavior) was characterized *in vitro*[5] and *in vivo*[6] indicating that self-propulsion promotes collective displacement, convection and mixing. In this study we developed urease-powered nanomotors based on biocompatible and biodegradable poly(lactic-co-glycolic acid) (PLGA) NPs, FDA and EMA approved,[7] as core. NMs were evaluated for active delivery of pDNA, which was integrated in the PLGA NMs using layer-by-layer (LBL) assembly.[8]

Delivery efficiency of fluorescently labeled PLGA NMs was evaluated in 2D (HeLa cells) and 3D (RT4 cells) cell cultures by flow cytometry and optical microscopy as a function of experimental parameters such as NMs and fuel concentration, and incubation time. Quantitative analysis demonstrated effective enhanced delivery in the presence of fuel. Furthermore, longer incubation times and higher concentrations of nanomotors led to higher delivery enhancements. Notably, two different cell viability measurements, colorimetric and metabolic, determined that cell viability remains unaffected in the range of NM's and fuel concentrations explored.

As a conclusion, the newly developed PLGA NMs proved to be a promising drug delivery system for pDNA delivery, especially when acting collectively as a swarm.

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POSTER 55 presented by:

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GROUP: Smart nano-bio-devices
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Magnetic navigation of swarms of enzyme-powered nanomotors with photothermal properties for immunogenic cell death

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Immunogenic cell death (ICD) is a process where damage-associated molecular patterns (DAMPs), such as ATP and calreticulin (CLRT), are released or exposed at the cell's surface. ICD has emerged as a promising strategy for enhancing the efficacy of cancer immunotherapy. Recent studies have demonstrated that ICD can be induced by means of light-triggered effects without the need for chemotherapeutics with potential side effects.[1] Light-responsive nanomaterials could improve ICD induction further if they could collectively displace and penetrate more efficiently into tumors. Advanced nanomaterials able to convert chemical energy into motion or nanomotors (NMs) are being actively explored due to their ability to overcome different biological barriers and their capability to collectively displace in the form of swarms.[2] Among these, urease-powered nanomotors have gained significant attention due to their biocompatibility, biodegradability, and the possibility of using urea as fuel at physiological concentrations to power their motion.[3] In addition, the use of iron oxide as chassis of these motors allows to combine the motion capabilities with photothermal and magnetic properties to offer an additional advantage for therapies.[4]

In this work we investigated the magnetic navigation capabilities of swarms of urease-powered iron oxide nanomotors (IONMs), guided by external magnetic fields, for enhanced and selective displacement and accumulation in desired regions of 3D-printed phantom models. In addition to their navigational abilities, we evaluated the photothermal properties of the iron oxide nanoparticles for induction of vapor nanobubbles (VNBs) formation upon irradiation with a pulsed laser to induce selective cell killing. As light-triggered cell death by means of VNBs holds the potential for generating DAMPs crucial to activating anti-tumor immune responses, we proceed to characterize the release of ATP and CLRT exposure in treated samples.

The combination of magnetic navigation and photothermal properties of IONMs proved to have clear potential for the selective displacement and accumulation of NMs and to induce the release and exposure of ICD hallmarks upon irradiation.

POSTER 56 presented by:

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Single-Molecule Analysis of Protein Corona Formation on Polymeric Assemblies

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Nanomedicine has gained considerable attention in the recent past, owing to the massive success of RNA-based vaccines within lipid nanoparticles.[1] Nanoparticle carriers, when rationally designed, can effectively protect cargoes from degradation and deliver them in a targeted and specific way. However, one of the primary challenges is understanding the fate of such nanoparticles when they are present in highly crowded physiological environments. When nanoparticles are injected into the bloodstream, it is presumed that they interact and get rapidly coated with a layer of adsorbed proteins, popularly termed as 'protein corona.' This corona formation can completely transform the nanoparticle characteristics and associated functions like targeting and therapeutic efficacy.[2] As a result, recent investigations on the nanoparticle-protein corona have focused on characterizing the composition of the corona and how parameters such as nanoparticle size, shape, and surface properties can modify it.[3] Polymeric nanostructures such as vesicles and micelles formed by spontaneous self-assembly of amphiphilic copolymers have been a topic of burgeoning research interest due to the strong affinity of their hydrophilic portions with water molecules, which create a strong repulsive steric potential that can prevent protein fouling and other unspecific interactions.[4] Investigations on the adsorption of proteins on polymer nanoparticles are still in their infancy, especially when such systems are incubated in a pure plasma medium. In the ongoing study, we look to address this gap, probe, and quantify corona formation of single protein molecules that are particularly present in blood plasma on different polymeric assemblies using a highly sensitive Fluorescence Correlation Spectroscopic technique.[5]

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POSTER 57 presented by:

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Generating Artificial Targets to Deliver Therapies Specifically to the Brain

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Treatment of neurological disorders such as Alzheimer's disease is hindered by the presence of the blood-brain barrier (BBB), a protective barrier composed of the specialized endothelial cells lining the brain vasculature. To overcome the BBB, current brain-delivery strategies bind nanoparticles to targets on the brain vasculature. However, such strategies have inherent brain-specificity limitations, as these 'natural' targets are also found in the peripheral vasculature, leading to off-target nanoparticle delivery to organs like the lungs and liver.

Here, we present a novel delivery strategy which exploits the specialization of the BBB to generate 'artificial' targets selectively on brain endothelial cells (BEC), thereby boosting brain specificity. We demonstrate the low-endocytic rate of BEC vs. peripheral EC may be harnessed to selectively retain free molecular tags on the surface of the brain vasculature, thereby acting as artificial targets to direct nanoparticles towards the brain with no increased accumulation in peripheral organs. We term our strategy Brain Targets Artificially Generated (Brain-TAG).

In addition, by screening peptide libraries, we probe the endocytic rate of individual cell-membrane components across different endothelial phenotypes to identify molecular tags suitable for the application of the Brain-TAG strategy. By identifying candidates for brain targeting based on their selective retention on the brain vasculature as opposed to their specific binding, our screening paradigm increases the molecular repertoire at our disposal to achieve therapy delivery with enhanced brain specificity.

POSTER 58 presented by:

NAME: Christopher James
GROUP: Biomaterials for regenerative therapies
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Biomaterial Incorporated Human Mesenchymal Stem Cell Secretome For Cardiac Regeneration

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The therapeutic effect of stem cell transplants for heart disease is now attributed to the pro-regenerative microenvironment created through secreted bioactive molecules such as growth factors (GF), cytokines, and extracellular vesicles known collectively as the secretome. Mesenchymal stem cell (MSC)-derived factors have been shown to protect the heart against hypertrophy or promote synchronous contraction.[1,2] To address the short duration of secretome activity, we aimed to incorporate it into a biomaterial for sustained release and enhanced cardiac regeneration.

To produce bone marrow-derived human MSC secretome, cells were cultured for 48 hours in low serum media under three different conditions: 2D and 3D normoxia (2D and 3D), and 2D hypoxia (Hyp.). A human cytokine array was carried out to determine the cytokines and GF levels, selected factors were quantified by ELISA. The secretome activity was tested on human umbilical vein endothelial cells (HUVEC) and human cardiac fibroblasts (hCFb). For encapsulation, we synthesised poly(lactic-co-glycolic acid) (PLGA) nanoparticles, using the water-oil-water emulsion method.

Several factors and cytokines such as angiogenin, VEGF, IL-6 and IL-8 were detected. ELISA analysis indicates that there was no difference in the levels of IL-6 between the secretomes. Higher levels of VEGF were detected in the secretome obtained by hypoxia. In cellular studies all three secretomes showed no cytotoxicity. Based on these results, we are currently encapsulating the secretome obtained from hypoxic treatment.

Acknowledgements

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POSTER 59 presented by:

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Targeting plasmid-encoded proteins that contain immunoglobulin-like domains to combat antimicrobial resistance.

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Antimicrobial resistance (AMR) poses a significant threat to human health. Although vaccines have been developed to combat AMR, it has proven challenging to associate specific vaccine antigens with AMR. Bacterial plasmids play a crucial role in the transmission of AMR. Our recent research has identified a group of bacterial plasmids (specifically, IncHI plasmids) that encode large molecular mass proteins containing bacterial immunoglobulin-like domains. These proteins are found on the external surface of the bacterial cells, such as in the flagella or conjugative pili. These plasmids frequenty include genetic elements that encode multiple AMR determinants. IncHI-encoded AMR can be present in enterobacteria such as Salmonella, Escherichia coli, Klebsiella pneumoniae and Citrobacter freundii. Plasmids of the IncHI2 subgroup predominate in antibiotic-resistant Salmonella isolates. In S. enterica serotype Typhi, more than 40% isolates harbor an IncHI plasmid. Of special concern is the recent report of an AMR clone of the highly virulent E. coli ST95 lineage. E. coli ST95 clones cause neonatal meningitis and sepsis. They are usually sensitive to several antibiotics. This AMR clone harbors an IncHI2 plasmid that carries, among other factors, genes encoding determinants of resistance to colistin and multiple other antibiotics (including the extended-spectrum beta-lactamase blaCTX-M-1 gene cluster). The spread of such an AMR ST95 clone could pose a threat to human health worldwide.

In this study, we show that these proteins are antigenic and can protect mice from infection caused by an AMR Salmonella strain harboring one of these plasmids. Furthermore, we successfully generated nanobodies targeting these proteins, which have been shown to interfere with the conjugative transfer of IncHI plasmids. Big proteins are also encoded in other groups of plasmids, such as IncA/C and IncP2. In a recent study, IncA/C plasmids accounted for 50% of all plasmids isolated from clinical K. pneumoniae strains harboring the blaNDM gene. Notably, the monophasic variant of S. Typhimurium, S. enterica serovar 4,[5],12:i:-, which has emerged as a global

cause of multidrug-resistant salmonellosis, predominantly harbors IncHI1 and IncA/C resistance plasmids.

Our study shows that targeting the plasmid-encoded Big proteins can be a valuable strategy in combating AMR infections caused by bacteria harboring different groups of AMR plasmids. Since the selected antigens are directly linked to AMR itself, the protective effect extends beyond specific microorganisms to include all those carrying the corresponding resistance plasmids.

POSTER 60 presented by:

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GROUP: Bioinspired onteractive materials and protocellular systems
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New concepts for synthetic cell membranes as a platform to interact with biology

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The fabrication of biomembranes that faithfully capture cell membranes' properties and dynamic functions remains a considerable hurdle in the progress towards synthetic cells and their application. In this work we introduce a new concept for synthetic cell membranes based on the self-assembly of zwitterionic Janus dendrimers and amphiphilic comb polymers. Their molecular design allows to tailor the hydrophobic/hydrophobic balance and program their self-assembly into vesicles known to us as dendrimersomes and ionic-combisomes. Their special organization afforded membranes that amalgamate the stability of classic polymersomes with the biomimetic thickness, flexibility, and lateral mobility of liposomes. Such unparalleled matching of biophysical properties and the ability to locally reconfigure the molecular topology of its constituents enable the harboring of functional components of natural membranes and even fusion with living bacteria to "hijack" their periphery. This provides an almost inexhaustible palette to design the chemical and biological makeup of these biomembranes resulting in a powerful platform for fundamental studies and technological applications. One an example of how these biomembranes can be utilize is to fabricate macrophage-mimetic phagocytic synthetic cells capable of eradicating viruses and antibiotic-resistant bacteria. The phagocytic synthetic cells consist of synthetic vesicles programmed to specifically recognize, engulf, and destroy pathogens by imitating nature's blueprints. The phagocytic synthetic cells may serve as a new paradigm to fight antibiotic-resistant bacteria and viral infections.

POSTER 61 presented by:

NAME: Fichna Kristin
GROUP: Smart nano-bio-devices
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Enzyme-powered nanomotors for enhanced drug delivery

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In recent years, enormous research efforts have been made to minimize the side effects of drugs and to increase their therapeutic efficiency in the treatment of cancer. Bladder cancer, for example, is the ninth most common cancer worldwide for which current therapies prolong patient survival, but also show high relapse rates, making it urgent to improve existing therapies. Using the catalytic reaction of enzymes that consume bioavailable fuels to propel micro- and nanoparticles (nanomotors) has expanded their potential applicability in nanomedicine and might provide a platform to overcome drug delivery challenges. Here, we present urease-powered nanomotors based on mesoporous silica nanoparticles (MSNP) loaded with clinically relevant drugs (Mitomycin, Erdafitinib) for the potential treatment of bladder cancer. The procedure of MSNP synthesis to obtain homogeneous particle size distributions and ensure proper pore opening for subsequent drug loading has been optimized. Furthermore, swarming behaviour in ionic and proteinaceous media has been tested in presence of different concentrations of urea (0 mM up to 300 mM). In addition, spectral flow cytometry as a novel tool to analyse particle delivery efficiency has been carried out with mouse bladder carcinoma cells (MB-49) after incubation (1 h, 4 h) with active and passive FITC-labelled nanoparticles at different urea concentrations. Furthermore, the nanomotors showed collective swarming behaviour in the tested media at concentrations higher than 40 mM urea. When the nanoparticles were incubated with MB-49 cells, active nanomotors showed a 3.6-fold increase of the delivery efficiency in presence of 100 mM urea compared to passive particles after only 1 h of incubation. The drug loading results and enhanced delivery efficiency of active nanomotors to MB-49 cells may proof their potential to be used in future nanomedical applications for the treatment of bladder cancer.

POSTER 62 presented by:

NAME: Cátia Lopes
GROUP: Molecular bionics
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Beyond the closed doors: unlocking novel therapeutic opportunities in Alzheimer's Disease

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Alzheimer's disease (AD) is the most common cause of dementia (60-80% of cases) with a prevalence near 50 million people. There is no cure available and there are only two classes of approved drugs that are effective in treat some of the cognitive symptoms and potentially delay the clinical decline. Despite the considerable progress achieved in the research of best performing disease modifying agents, the main clinical challenge remains the accomplishment of an efficient and safe therapeutic option that can arrest the disease progression and prevent cognitive failure.

One crucial aspect of AD is the accumulation of Amyloid-β (Aβ) plaques, which disrupt neuronal function and contribute to cognitive decline. Gene therapy presents an opportunity to modulate the expression of genes involved in Aβ metabolism, clearance, and degradation and, thus, help to reduce Aβ burden and slow disease progression.

Here we propose an innovative approach to improve AD pathophysiology by targeting the brain endothelium to modulate the expression of crucial genes responsible for boosting Amyloid-β (Aβ) clearance. By combining the power of super-selective binding strategies of nanocarriers with gene therapy, we create a unique opportunity to target and modulate the brain endothelium in the context of AD. Ultimately, our strategy will contribute to overcome the impaired Aβ transendothelial clearance, potentially halting disease progression and preventing cognitive decline. This work has the potential to make a significant global impact by introducing a novel approach that could transform the clinical course of AD pathogenesis.

POSTER 63 presented by:

NAME: Maria Jose Lopez Martinez
GROUP: Bioinspired onteractive materials and protocellular systems
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Kill&Repel Coatings: The marriage of Antifouling and bactericidal properties to mitigate and treat wound infections

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Wound infections originate when exogenous or endogenous bacterial pathogens are able to invade the wound bed. Bacterial colonization results in inflammation, delay of the healing process, and the risk of dissemination to other tissues. Together with the ever-increasing prevalence of drug-resistant infections, this represents a major threat to human health. Current antimicrobial strategies fail to avert this threat as they are unable to prevent initial bacterial attachment. Moreover, their antimicrobial activity is short-lived as both human cells and residues of killed bacteria accumulate on the surface blocking the bactericidal activity. Faced with these challenges, we developed the Kill&Repel coating strategy, which provides simultaneous bactericidal and antifouling properties. The coating consists of synthetic-natural water-soluble macromolecules that self-assemble on the surface forming antifouling polymer brushes functionalized with endolysins. These enzymes exhibit high specificity for killing bacteria and a low probability of generating bacterial resistance. The antifouling hybrids consist of N-(2-hydroxy-propyl)methacrylamide polymer grafted from the Liquid Chromatography Peak I (LCI) peptide. Bactericidal hybrids were generated by fusing LCI with different highly active types of endolysins that specifically target *S. agalactiae*, *S. epidermisdis*, *S. aureus*, and MRSA. The Kill&Repel coating was formed by the physisorption of the two hybrids from a dilute aqueous solution onto the surface of wound dressings. The coated dressings showed a drastic reduction in bacterial adhesion, thus diminishing the risk of infection. In addition, these dressings were able to reduce the concentration of planktonic bacteria (OD550=0.8) by over 92%, far more efficiently than free LCI-endolysin (50%). Furthermore, Kill&Repel modified dressings were able to completely eradicate bacteria in a simulated infection without allowing residues to adhere to the surface. This demonstrates a self-cleaning mechanism with inexhaustible antimicrobial activity.

The Kill&Repel coating provides a simple and universal means of imparting specific antimicrobial activity to a wide range of medical devices and implants, holding promise for clinical application.

POSTER 64 presented by:

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GROUP: Bacterial infections: antimicrobial therapies
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Novel insights into the regulation of the nrdAB class Ia ribonucleotide reductase from *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a highly adaptable opportunistic pathogen, exhibiting both acquired and innate antibiotic resistance mechanisms. The identification of novel therapeutic strategies is essential due to its ability to survive in diverse environments. Ribonucleotide reductases (RNRs), essential enzymes responsible for dNTPs synthesis, have emerged as promising targets to combat *Pseudomonas aeruginosa* infections. Among these, the class Ia RNR, encoded by nrdAB operon, is regulated by different factors such as NrdR and AlgR. However, there are still certain gaps in our understanding of the regulatory network of nrdAB operon.

Experimental and bioinformatic analysis have described a long 5'UTR (untranslated region) on the nrdA promoter, suggesting that the 5'UTR may play a crucial role in the nrdAB operon regulation. Experimental analysis indicated that the absence of the 5'UTR leads to increased nrdAB expression, in contrast to its regulatory effect on other genes such as rpoD and nrdJab. Bioinformatic analysis suggested that 5'UTR may contain a B12 riboswitch or a small non-coding RNA (sRNA). Although the first hypothesis has not been experimentally validated, the second hypothesis appeared plausible, considering the diverse functions of sRNAs, including mRNA stability and half-life. A transcriptional shut-off assay demonstrated that the presence of 5'UTR leads to a reduction in nrdA mRNA half-life.

Further research is required to fully elucidate the intricate mechanisms involved in the transcriptional and post-transcriptional regulation of the nrdAB operon.

Acknowledgements

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POSTER 65 presented by:

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MR1 protein: the gateway to target Tuberculosis infection

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Globalisation makes infectious disease outbreaks and their rapid spread one of the major challenges for public health. The over and misuse of antibiotics, the incapacity of low-income countries' healthcare systems to effectively handle patients' needs, and the lack of new drugs, leads to the continuous emergence of new drug and multidrug-resistant bacteria. Mycobacterium tuberculosis (Mtb), the causative agent of Tuberculosis (TB) that devastated Europe during the first half of the 19th century, is still responsible for the death of over 1.6 million people annually, being the second deadliest infection after COVID-19. We have previously described that polymeric nanoparticles containing isoniazid and clofazimine presented lack of toxicity, dose-dependent response, and improved therapeutic efficacy when compared to free drugs *in vivo* [1]. However, the site-specific delivery of drugs to infected cells remains elusive. Human T cells can recognise several protein and non-protein antigens produced by bacteria during infection. Among T cells, a distinct subset population known as Mucosal-associated invariant T (MAIT) cells exhibit the capacity to specifically recognise bacterial-derived metabolites (including from Mtb) presented by MHC class I-like related (MR1) protein. MR1 is an evolutionarily conserved protein that mainly resides in the endoplasmic reticulum of host cells but is readily translocated to the cell surface upon metabolite binding and association with β 2 microglobulin (β 2m). This translocation enables MR1 to function as a vigilant sensor for intracellular infection. Using phage display technology, we screened for peptides that bind specific MR1-metabolite complexes to directly target antibiotic-loaded polymersomes to infected cells. We have expressed and purified MR1 Ectodomain-β 2m complexes with and without acetyl-6-formylpterin (Ac-6-FP), a synthetic analogue of 6-formylpterin (6-FP). 6-FP is an intermediate in folate biosynthesis pathways found in bacteria which has been shown to increase MR1 expression in the plasma membrane of cells *in vitro*. These purified complexes have been used as a target for phage display experiments. In addition, we are also using THP-1 cells exposed to bacterial supernatants for the phage display selection. The lead peptide binders will be used to functionalise polymersomes and assess binding and internalisation in infected and non-infected cells *in vitro*.

POSTER 66 presented by:

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GROUP: Nanoscale bioelectrical characterization
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Probing the electrical properties of isolated fibers from cable bacteria without contacting them by scanning dielectric microscopy

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Electron transport in cable bacteria take place along a network of fibers embedded in their cell envelope. The fibers are typically 50 nm in diameter and several microns long. Measuring the electron transport properties along these tiny fibers poses important challenges associated to the need of physically contacting them either with planar microelectrodes or with scanning probe electrodes. Recently, it has been shown that Scanning Dielectric Microscopy can access the electrical properties of isolated fibers from cable bacteria without physically contacting them.[1] In Scanning Dielectric Microscopy (SDM) one first measures the long-range electrical forces acting on a sharp conducting probe located in close proximity to the isolated fiber in response to an applied electric potential, and then quantifies these forces by using finite element numerical calculations. The result of the measurement is the complex permittivity of the fibers, which includes both the dielectric constant and the conductivity of the fibers. In the present communication we describe this method and analyze the variety of properties that can be obtained from this type of studies, which include information on the internal structure of the fibers or on any anisotropy existing in the conduction properties.

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Keywords: Cable Bacteria, Scanning Dielectric Microscopy, Electrical properties, isolated fibers

POSTER 67 presented by:

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Design and characterization of bifunctional hydrophilic Janus micelles as novel nanodrugs.

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Janus micelles are featured by their biphasic geometry of heterogeneous composition and distinctive properties in the core and corona. Such anisotropic design has attracted much attention from the scientific community due to the versatility of chemistries employed for generating Janus' morphologies and their potential applications. In this work, we present a novel ABC amphiphilic triblock copolymer system with the ability to form Janus micelles. The micelles are generated by solution-mediated self-assembly of the A and C hydrophilic, and B hydrophobic blocks. Subsequently, the hydrophilic blocks in the proposed triblock system will be functionalized with different and specific bioactive ligands to enable the constitution of multifunctional supramolecular scaffolds. The final aim of the project herein presented is to develop nanodrugs with well-defined dissimilar phenotypical domains, in the same fashion as the asymmetric functionalisation of antibodies that already exist in nature, to perform alike. To this end, poly(ethylene glycol)-polylactide-poly(N-vinylpyrrolidone) PEG-PLA-PVP triblock copolymer was synthesised in two steps: first, poly(ethylene glycol)-polylactide-2-bromo-2-methylpropanoate (PEG-PLA-Br) diblock macroinitiator was synthesized by the ring-opening polymerization of DL-lactide with commercial poly(ethylene glycol) and quenched with 2-bromo-isobutiryl-bromide. After that triblock copolymer was 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). Such techniques enabled us to verify the controlled radical polymerization of NVP and the complete conversion of PEG-PLA-Br diblock to the proposed PEG-PLA-PVP triblock. The morphologies adopted by PEG-PLA-Br and PEG-PLA-PVP in solution were investigated by transmission electron microscopy (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. Such differences indicate the asymmetric distribution of both hydrophilic blocks, PEG and PVP, in the self-assembling of the PEG-PLA-PVP triblock system. Further structure characterizations will include atomic force microscopy (AFM), cryo-TEM, electron energy loss spectroscopy (EELS), and ultimately the functionalization of both coronas with different targeting ligands.

POSTER 68 presented by:

NAME: Anna Panteleeva
GROUP: Nanobioengineering
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Neurofilament light biosensor in a brain-on-a-chip for neuronal activity monitoring

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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, and the vast majority of NDD candidates do not advance to the last phase in drug discovery. One of the main limiting stages of drug discovery is the crossing of the blood-brain barrier (BBB), which is considered one of the most highly protective membrane of the body, impeding many toxins and drugs to enter the brain. Its dysfunction has been linked to several NDDs.

Traditional animal models have been instrumental in advancing our understanding of NDDs and drug delivery, but they often fail to fully mimic the complexity of human neural responses. The need for innovative and more precise tools to study neuronal activity and develop effective therapeutic interventions is relevant as ever. Brain-on-a-chip (BoC) technology has recently emerged as a powerful tool to study neural networks' behavior in a controlled environment. The incorporation of a BBB component in a BoC further enhances its potential as a robust and physiologically relevant model and allows to better mimic the BBB's unique properties. Most of the reported works on BoCs are based on the traditional systems with optical methods of detection, such as microscopy, but they lack the ability to continuously monitor, are time-consuming, and moreover, require expensive equipment and trained staff. Electronic monitoring systems, such as electrochemical biosensors, offer many advantages including the possibility to automatically monitor a wide range of analytes and biomarkers for personalized disease study or drug testing in NDDs.

In this context, we present the development of a neurofilament light (NfL) biosensor that provides a non-invasive method for continuous neural activity monitoring via NfL sensing. NfL is a protein excreted from neurons to the extracellular matrix, into the cerebrospinal fluid and bloodstream, following axonal damage or degeneration of neurons. The NfL is a sensitive biomarker for various neurological disorders.

The biosensor we are currently using consists of a commercial screen-printed electrode and a sandwich immunoassay system for the detection of NfL. This electrochemical immune sensor has two different configurations: a) a label-free impedance sensor and b) a labelled amperometric immunosensor. This approach offers several advantages

over the traditional methods with optical detection, such as lower cost, easier integration with microfluidics, higher sensitivity, faster response time, and lower interference from background signals.

The next step in our research would be to monitor the NfL produced in a 3D neuronal co-culture with the appropriate cells (endothelial, pericytes and astrocytes) for the construction of the BBB, and to test our biosensor in close-to-real conditions.

POSTER 69 presented by:

NAME: Marina Placci
GROUP: Targeted therapeutics and nanodevices
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Transcytosis of anti ICAM-1 NPs in a transwell-model of the lung endothelium

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Types A and B Niemann-Pick disease (NPD) is a lysosomal storage disorder that mainly affect the central nervous system and the lungs, respectively. *In vivo* experiments have shown that both organs can be targeted by polymer nanoparticles (NPs) coated with anti-intercellular adhesion molecule 1 (ICAM-1) and loaded with NPD therapeutics. ICAM-1 is transmembrane glycoprotein capable of mediating transcytosis of NPs through the blood-brain barrier for brain delivery of NPD-A therapeutics. However, ICAM-1-mediated transcytosis across the lung endothelium has never been studied, although this is fundamental for NPD-B therapeutics to reach other pulmonary cells. Thus, in this study, transcytosis of anti-ICAM-1 NPs was investigated using transwell models consisting of an apical (AP) chamber, a membrane seeded with lung endothelial cells and a basolateral (BL) chamber containing lung epithelial cells. Affinity, binding, uptake, and transcytosis were examined using radiotracing and fluorescence microscopy. Anti-ICAM-1 affinity had a maximum binding of 8.2x106 Ab/cell and a dissociation constant of 73.59nM. Model polymeric anti-ICAM-1 NPs were 220.9±2.8nm in diameter, 0.18±0.01 polydispersity index, and -19.5±0.4mV ζ-potential. They showed specific targeting of ICAM-1 expressing cells and also compared to control IgG NPs. Anti-ICAM-1 NPs targeted lung endothelial cells and were internalized by them. With time, endothelium-associated NPs decayed with a concomitant increase in the number of NPs transported to the BL chamber, demonstrating transcytosis across the barrier. Moreover, anti-ICAM-1 NPs transported across the lung endothelium were internalized by the underlying lung epithelial cells, for which this pathway may be used to deliver therapeutics to these cells. Altogether, these data indicate the suitability of this strategy to deliver medicines aiming to treat NPD-B, as previously reported for NPD-A, and likely other diseases affecting the lungs, such as pulmonary cancers.

POSTER 70 presented by:

NAME: Carles Prado
GROUP: Smart nano-bio-devices
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Designing Enzymatically-Powered PLGA Nanobots and Exploring its Swarming Behavior for Skin Applications

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Skin possesses unique advantages as an administration route for drug delivery. Nevertheless, the stratum corneum is a formidable barrier to overcome before spreading to deeper layers. Topical formulations based on nanoparticles take advantage of the hair-follicle route as a natural entry door.[1] Due to the low density of these appendages and the presence of sebum (protective oil), a lot of the dose is lost on the skin's surface.[2] Here, we present the synthesis and characterization of a new urease-nanobot based on an organic biocompatible and biodegradable chassis of PLGA/Chitosan (FDA approved materials). Studying its collective behavior, it was demonstrated how in the presence of the fuel, nanobots are able to expand more and explore further areas, if compared with passive controls. Consequently, this movement could be used for reaching more hair follicles. However, this entry door is commonly hampered by sebum. We have seen how the catalytic reaction of our nanobots provokes the mixing of an oil/aqueous interface, displacing upwards the remaining oil phase. That allows the nanobots to cross this barrier more easily. This phenomenon could be explored for common diseases such as acne, which is characterized by an overproduction of sebum. With all, nanobots have a huge potential for topical delivery, but further studies are required for better understanding how enzymatic-nanobots would behave on skin surfaces and in biological models.

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POSTER 71 presented by:

NAME: Lucia Roman
GROUP: Nanomalaria
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Nanotechnological Approaches against Leishmaniasis: Aerosol Therapy with Pentamidine-loaded Liposomes

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Leishmaniasis is one of the most important neglected tropical diseases. However, only four drugs are currently in use, which have significant side effects and a long and painful administration regimen, either intramuscular or intravenous. This is the case of pentamidine, the drug of choice in many regions of South America for a form of the disease called mucocutaneous leishmaniasis. Our aim was to test the potential of phospholipid vesicles to improve patient compliance and the efficacy of this drug for the treatment of leishmaniasis by aerosol therapy. Pentamidine-containing liposomes were coated with chondroitin sulfate, heparin or left uncoated. Liposomes reduced the cytotoxicity compared to free pentamidine and, when coated, the effect on L. infantum and L. pifanoi promastigotes was significantly improved, as well as its uptake by macrophages. In an attempt to explore a less invasive route of administration, we carried out an evaluation of liposome nebulisation using the Next Generation Impactor, which mimics the human respiratory tract. The fine particle fraction, i.e. the amount of drug that potentially reaches the deeper airways of the lung, is significantly higher when pentamidine is encapsulated (~68%) than when it is not (~53%), and the mean diameter is reduced to a range between 1.4 and 1.8 µm, suggesting a higher deposition of the nebulised liposomal preparations on the lung alveoli. This work represents a promising advance in the formulation of antileishmanial drugs for optimised delivery.

POSTER 72 presented by:

NAME: Alessandro Ronzoni
GROUP: Molecular bionics
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Live Cell Imaging Of LRP1 Trafficking

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LRP1 is a member of the LRP family, a group of receptor proteins involved in lipid homeostasis, cell migration, proliferation, and differentiation. LRP1 is involved in neurodegenerative disorders and cancer; thus, it is crucial to understand its structure and interactions with ligands (1).

Part of my research project employs molecular biology techniques to engineer human LRP1 DNA, express it in various cell lines, and perform imaging on live cells.

Working with a plasmid coding for the sequence of full-length human LRP1, I generated a fusion protein with the red fluorescent protein “mCherry XL.” The obtained DNA sequence, called “LRP1-mCherry XL”, codes for LRP1 fused to mCherry XL at its C-terminal cytosolic domain, and the features present in the vector DNA sequence make it suitable for expression in human cell lines.

I am using this plasmid to transfect HEK293T and HBMEC cells and achieve fusion protein expression. With this tool, I perform confocal imaging, observing a successful presentation of the protein and its localization on the cell membrane. So far, I have completed multi-channel acquisition, observing the cell nuclei, the cell membrane, and the LRP1-mCherry XL protein. The obtained data consists of 2D images, 3D reconstructions, and videos.

Especially the video files show how the protein is not statically expressed on the membrane but translocates within the cell. Starting from this data, we decided to co-express it with syndapin2, already reported to mediate the transcytosis of Amyloid-β together with LRP1 (2). We aim to have both proteins fused with fluorophores, enabling us to observe the behaviors of the two proteins, their colocalization, and the dynamic of

the tubulation process. Once obtained this data, we aim to introduce labeled Amyloid-β in the experiment and simultaneously observe all three elements during the transport process.

This project aims to collect data regarding the behavior and dynamics of LRP1 during the trafficking process; as a side project, I am expressing and purifying LRP1 to study its structure. With the data from these two experimental lines, we hope to contribute to understanding this protein's characteristics, its role in intracellular transport, and its mechanisms of interaction.

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POSTER 73 presented by:

NAME: Noelia Ruiz-González
GROUP: Smart nano-bio-devices
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Swarms of enzyme-powered nanomotors enhance the diffusion of macromolecules in viscous media

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In recent decades, nanotechnology has made significant progress in drug delivery systems. The goal is to improve therapy effectiveness by precisely releasing drugs to specific tissues. However, there are still challenges to overcome. One major challenge is the presence of biological barriers, such as viscoelastic fluids like synovial fluid in joints, which mainly contain hyaluronic acid. The complex network of these fluids hinders the transportation of nanosystems, causing conventional particles to get trapped and limiting their ability to reach the target area. Therefore, there is a need for innovative technologies that can enhance the delivery of therapeutic agents. To overcome the obstacles presented by complex media, one promising approach is the development of "active" nanoparticles or nanomotors (NMs). However, the exploration of enzyme-powered nanomotors capable of navigating and influencing viscous fluids is still in its early stages. These enzyme-powered nanomotors offer great potential, as their coordinated movement can be driven by enzymatic reactions, effectively utilizing the biofuels present in the human body. Furthermore, some of these enzymatic nanomotors can modify the characteristics of the extracellular matrix by reducing its viscosity, thus facilitating improved diffusion of therapeutic agents. In this study, we introduce a nanotechnological strategy using two swarms of nanomotors, namely hyaluronidase NMs (HyaNMs, Troop 1) and urease NMs (UrNMs, Troop 2), which synergistically enhance the diffusion of macromolecules within the synovial fluid. Troop 1 demonstrates the capability to break down the intricate network of synovial fluid, both *in vitro* and ex vivo, thereby reducing its viscosity. This, enables Troop 2 to navigate more effortlessly through the viscous media. Moreover, the collective movement of Troop 2 significantly enhances the diffusion of Dextran macromolecules. These findings offer promising prospects for utilizing enzyme-powered NMs in the treatment of joint injuries, augmenting therapeutic effectiveness, and facilitating faster and more efficient delivery of therapeutic agents compared to conventional approaches.

POSTER 74 presented by:

NAME: Tiziana Russo
GROUP: Bioinspired onteractive materials and protocellular systems
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Super predatory ionically-linked comb polymers for engulfment of micro and nanoparticles

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Endocytosis is a fundamental process that plays a pivotal role in numerous biological phenomena such as nutrient uptake, cellular signaling and immune response. Therefore, the development of cell membrane mimics capable of encapsulating particles and pathogens through engulfment represents a breakthrough in the fields of healthcare, biotechnology and environmental restoration. Several systems have been investigated for this purpose, but all of them come with some limitations: liposomes show limited mechanical stability which can lead to rupture of the membrane while polymersomes, due to their high molecular weight, require higher adhesion energies for engulfment to proceed. To overcome these constraints, we developed an innovative system obtained by self-assembly of amphiphilic ionically-linked comb polymers (iCPs). The non covalent bond between the hydrophilic backbone, consisting of poly(carboxybetaine acrylamide-co-N,N-dimethylaminopropyl acrylamide) (poly(CBAA-co-DMAPAA)), and the hydrophobic tails, made of didodecylhydrogen phosphate (DDP), determines high flexibility of the i-combisomes membrane and its ability to locally reconfigure. These unique characteristics allow for engulfment to happen spontaneously without needing external energy inputs.

The engulfment of silica and polyethylene particles was assessed through confocal laser scanning microscopy, cryo-TEM and dynamic light scattering. The results show that i-combisomes exhibit the ability to engulf micro and nanoparticles efficiently exclusively relying on physical interactions. In addition, i-Combisomes were tested as a mean to segregate microplastics and they proved themselves to be a promising system for environmental remediation.

POSTER 75 presented by:

NAME: Daniel Sánchez de Alcázar Melendo
GROUP: Smart nano-bio-devices
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Chopping off urease into two: trimers as active matter for nanomotors

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Urease, a metalloenzyme widely found in bacteria, plants, and fungi, plays a crucial role in nature, catalyzing the hydrolysis of urea into ammonia and carbamate, providing a nitrogen source for many organisms. The urease-catalytic reaction has been extensively studied due to its implications in various fields, such as agriculture, medicine, and environmental sciences. In the last few decades, advancements in nanotechnology have paved the way for harnessing the power of enzymes through their integration with nanoparticles, endowing them self-propulsion features. Among these enzymes, urease has gained significant prominence due to its intrinsic properties, i.e., high turnover number and small substrate and byproduct sizes which contribute to enhanced motion properties.

Recent studies have shown that the trimeric form of urease preserved catalytic activity and stability, raising question about the role of the hexameric form, the potential utilization of the trimer in the nanomotors design and shed light on the mechanism underlying the motion of nanomotors. Understanding the functional significance of different oligomeric states of urease is crucial for unraveling their roles in biocatalysis and exploring their potential applications in designing a new generation of nanomotors.

Here, we present a preliminary demonstration showcasing not only the preservation of catalytic properties in the trimeric form of urease but also its ability to exhibit motion at the single particle level and swarm behaviour. Furthermore, we propose strategies to enhance trimer production through the utilization of chemical and molecular biology tools to induce the dissociation of the hexameric enzyme into its trimeric form.

In the first strategy, we suggest exposing the enzyme to detergents or reducing agents, which could potentially destabilize the hexameric form. The introduction of these agents may disrupt the interactions holding the hexamer together, leading to trimer formation.

In the second strategy, we propose a theoretical approach involving the destabilization of the interface contact by introducing mutations in key amino acids involved in the interaction. By targeting specific amino acids responsible for the hexameric assembly, we can potentially promote the dissociation of the hexamer.

These strategies present promising avenues for increasing trimer production and can be explored further to optimize the production process of the trimeric urease enzyme.

POSTER 76 presented by:

NAME: Ramona Santini
GROUP: Nanoprobes and nanoswitches
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Drug Loading Strategies For Discotic Amphiphile Supramolecular Polymers In Water

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BTA-based supramolecular polymers are interesting systems for medical applications because of their high dynamicity and stimuli responsiveness in water[1]. Recently, IBEC developed a new class of BTA-based supramolecular polymers which showed responsiveness to temperature, salt concentration, pH, and light[2,3]. This versatility makes this new class of self-assembled fibers appealing for drug delivery purposes. In this work, we explored two strategies to incorporate different biologically active ligands into these polymers and demonstrate their employability as light-driven drug delivery systems. In the first strategy, we used a co-assembly approach in which two new discotic BTA-azo-monomers assemble forming the final helicoidal supramolecular fibers. In the second one, we decided to cage Iperoxo-azo[4], a potent photoswitchable derivative of the mAChR agonist Iperoxo[4]. Here, the interaction is based on the stacking between the azobenzene units of the ligand and the monomers. From the first approach, we obtained satisfying co-assemby results which were evaluated by transmission electron microscopy. Remarkably, the second system showed light-dependent biological effects in calcium imaging experiments on cells overexpressing M1 mAChRs. While caged Iperoxo-azo did not evoke significant changes, UV pre-illuminated fibers caused an increase in intracellular calcium levels because of the activation of M1 mAChRs by the uncaged ligand. These results suggest that the new class of BTA-based supramolecular polymers3 can potentially be used as light-driven drug delivery system for small, planar and amphiphilic drugs.

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POSTER 77 presented by:

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GROUP: Nanoscale bioelectrical characterization
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Nanoscale Multimodal Characterization of Operating Electrolyte-Gated Transistors

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Electrolyte Gated Transistors (EGTs) are emerging bioelectronic platforms that efficiently couples ionic and electronic transport processes to enable key technologies ranging from ultra-sensitive electronic biosensors to advanced neuromorphic devices. An electrolyte environment facilitates ionic-electronic coupling; however, it simultaneously confers major complexity in understanding the physics of these devices due to associated secondary effects such as material swelling and softening. Consequently, there is a demand for advanced characterization techniques capable of simultaneously probing multiple physical properties, including morphology, mechanical behaviour, and electrical properties, in operating EGT devices. However, existing methods have generally been limited to investigating one property at a time, and the availability of multimodal tools, particularly at the nanoscale, is scarce. Precisely addressing this critical gap, we developed multimodal characterization methods based on scanning probe microscopy to simultaneously probe the nanoscale morphological, mechanical and electrical properties in operating EGTs, offering novel insights into the physics of these devices and expanding our fundamental understanding.

POSTER 78 presented by:

NAME: Maria Jose Ugarte
GROUP: Biosensors for bioengineering
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Development of a Label-Free Plasmonic Biosensor for Accurate Diagnosis of Myasthenia Gravis

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Myasthenia Gravis (MG) is an autoimmune disease characterized by autoantibodies targeting the neuromuscular junction, particularly the acetylcholine receptor (AChR). Current clinical assays lack the ability to differentiate the diverse pathogenic mechanisms underlying MG. Complement C5b9 activation represents a particularly aggressive mechanism leading to motor plate disappearance. To address this limitation, we developed a novel plasmonic biointerface integrated with 2D and 3D models, enabling label-free detection of AChR autoantibody-mediated complement activation for rapid and accurate MG diagnosis.

The optimized biofunctionalization of cys-Protein G on a localized surface plasmon resonance (LSPR) gold surface facilitated oriented antibody binding and enhanced AChR autoantibody detection sensitivity. Comparative analysis with ELISA revealed a Limit of Detection (LOD) of 3.18 ng/mL. Further improvements were achieved by establishing a standard curve, resulting in a significantly enhanced LOD of 0.153 ng/mL.

Moreover, our plasmonic biosensor facilitated the monitoring of autoantibodies released into the supernatant after muscle damage in 2D cell cultures, shedding light on specific MG pathogenic mechanisms. Accurate detection and monitoring of AChR autoantibodies and complement-mediated activation demonstrated the potential of our biosensor as a powerful diagnostic tool for MG.

The label-free plasmonic biosensor holds great promise for future Point-of-Care (POC) and portable devices in precision medicine. It offers detailed insights into individual-specific pathogenic mechanisms underlying MG, contributing to accurate diagnosis and 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 precision medicine.

POSTER 79 presented by:

NAME: Akhil Venugopal
GROUP: Molecular bionics
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Dynamic Lipid Vesicles: Next-Generation Drug Delivery Systems

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Living systems possess a remarkable characteristic of operating away from equilibrium through metabolic processes involving both catabolic and anabolic reactions, i.e., the synthesis and breakdown of molecules by consuming chemical energy [1]. These out-of-equilibrium (OOE) processes often give rise to highly dynamic self-assembled structures that play a crucial role in defining fundamental characteristics of life, such as spatiotemporal control and adaptivity. Taking inspiration from the impressive power exhibited by living systems, there has been a growing interest in creating artificial out-of-equilibrium systems having a lifetime of their own [2]. However, studies and applications of self-assembled nanomaterials in a non-equilibrium steady state (NESS) where materials exist by the constant formation and degradation of nanostructures are unexplored. Here, we present the formation of dynamic lipid vesicles in NESS through chemical fuel-driven self-assembly. We have designed and synthesized a hydrophilic phospholipid mimic with a terminal aldehyde group and a hydrophobic tail with a terminal amino group. In the presence of an amino ester fuel, the hydrophilic aldehyde is converted into an imine with a lipid-like amphiphilic structure which eventually self-assembled into lipid vesicles. Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM) and Light Sheet Microscopy (LSM) analysis confirmed the formation of vesicles. The hydrolysis of the amino ester by lipase enzyme resulted in the disassembly of vesicles. The morphological evaluation of the system revealed higher-order aggregates that corroborated our initial hypothesis of vesicle disassembly. These dynamic vesicles are sustained only by a constant supply of fuel. In addition, by varying the concentration of the lipase enzyme we could shift the lifetime of the vesicles from hours to minutes. We aim to prove that these lipid vesicles in NESS can dynamically reconfigure with the cells to provide adaptive cellular contacts and hence can be used as an adaptive interface for targeted drug delivery.

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POSTER 80 presented by:

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Integrating phenotypic targeting in physiologically-based pharmacokinetics modeling

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Selective drugging, popularized as the "magic bullet", refers to the concept that drugs could specifically target molecules, cells, or biotargets of interest while minimizing interactions with other body parts. We have created a new method for selectively targeting cells using multivalent units that carry a mix of ligands and hydrophilic polymers. This approach reduces non-specific interactions and adjusts the binding strength of each ligand to favor entropy-driven association. By doing this, we engineer interactions that only occur with cells that express the corresponding combination of receptors, allowing us to target specific phenotypes of cells.

Here we are integrating phenotypic targeting within physiologically-based pharmacokinetics modeling (PBPK) to mimic the distribution of NPs in organs to help us define a proper administration strategy. The PBPK is built based on the circulation system and anatomy data to predict the distribution of the NPs into different organs. The association constant/affinity kA/j is derived from the phenotypic association theory to reveal the selectivity of NPs to different cells—the difference in kA/j results in a larger discrimination in distribution. An agent-based model was applied to simulate the adsorption, diffusion, metabolism, and elimination (ADME) of NPs in the cell. Through this model and the experiment *in vivo*, we obtain the diffusion rate to different organs, selectivity of NPs to different cells, and clearance rate. The prediction of NPs distribution with different administration strategies could be obtained based on the parameters to help design more effective and targeted therapies.

POSTER 81 presented by:

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YAT2150 alters *P. falciparum* protein homeostasis: towards a new family of antimalarial drugs.

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All *Plasmodium falciparum* stages have abundant intracellular protein aggregation, which is predicted to have a functional essential role for the pathogen. The inhibition of protein aggregation in malaria parasites has been recently proposed as a novel mode of action for new drugs. This mechanism presumably targets multiple proteins, which will likely prevent a rapid resistance evolution, as opposed to most other current antimalarial drugs which target products of one or a few genes. One such compound that interferes with protein aggregation, the bis(styrylpyridinium) salt YAT2150, a β-sheet intercalator, has a number of additional properties that make it a promising antiplasmodial agent, namely: (i) it is a fast-acting drug without cross resistance to chloroquine and artemisinin; (ii) it fluoresces when interacting with its molecular targets in Plasmodium cells, which makes it a potential theragnostic agent; (iii) its *in vitro* IC50 is below 100 nM against *P. falciparum* asexual blood stages and has a similar potency on early and late stage gametocytes; (iv) it belongs to an unexplored chemical family where no other antimalarial has been described up to date, which will prevent the adaptation by the parasite of preexisting resistance mechanisms to currently used drugs; (v) inability to select resistant mutants *in vitro* after 60 days of incubation, which postulates this compound as an 'irresistible' antimalarial drug deserving attention in a likely future scenario of widespread resistance to artemisinin; (vi) its synthesis is easy and rapid (only two steps), resulting in an attractive activity/cost ratio; and (vii) a long shelf life (months) at room temperature. We have characterized the PK/PD profile of YAT2150, and our current efforts are focused on widening its therapeutic window through the synthesis of chemical derivatives of lower toxicity and higher antiplasmodial activity, and their encapsulation in targeted nanocarriers. To conclude, we will present our latest proteomics results pointing at the functionality of protein aggregation in *P. falciparum* and our current hypothesis regarding why and how its inhibition can be lethal for the malaria parasite.

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