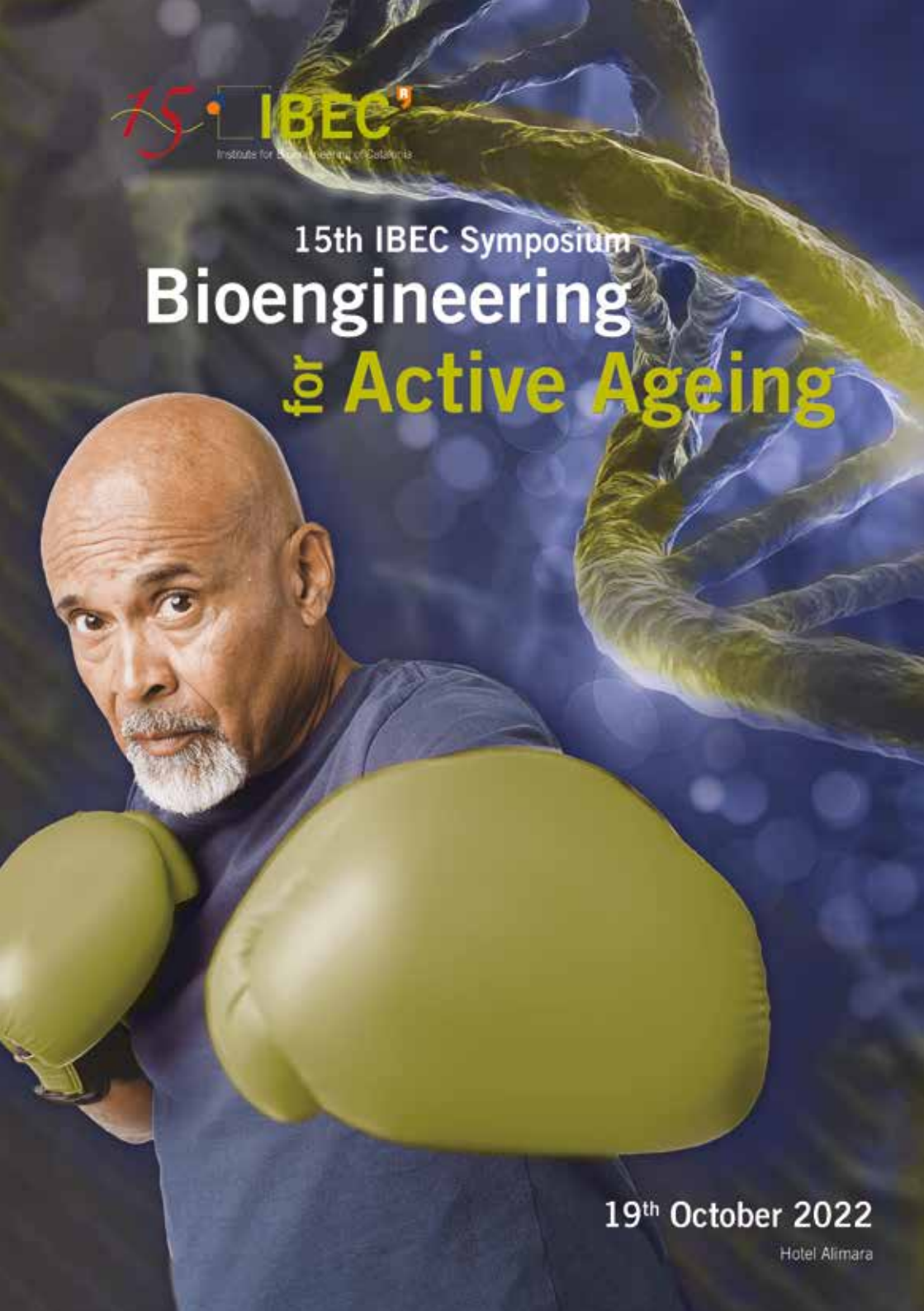
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15th IBEC Symposium
Bioengineering
for Active Ageing
19th October 2022
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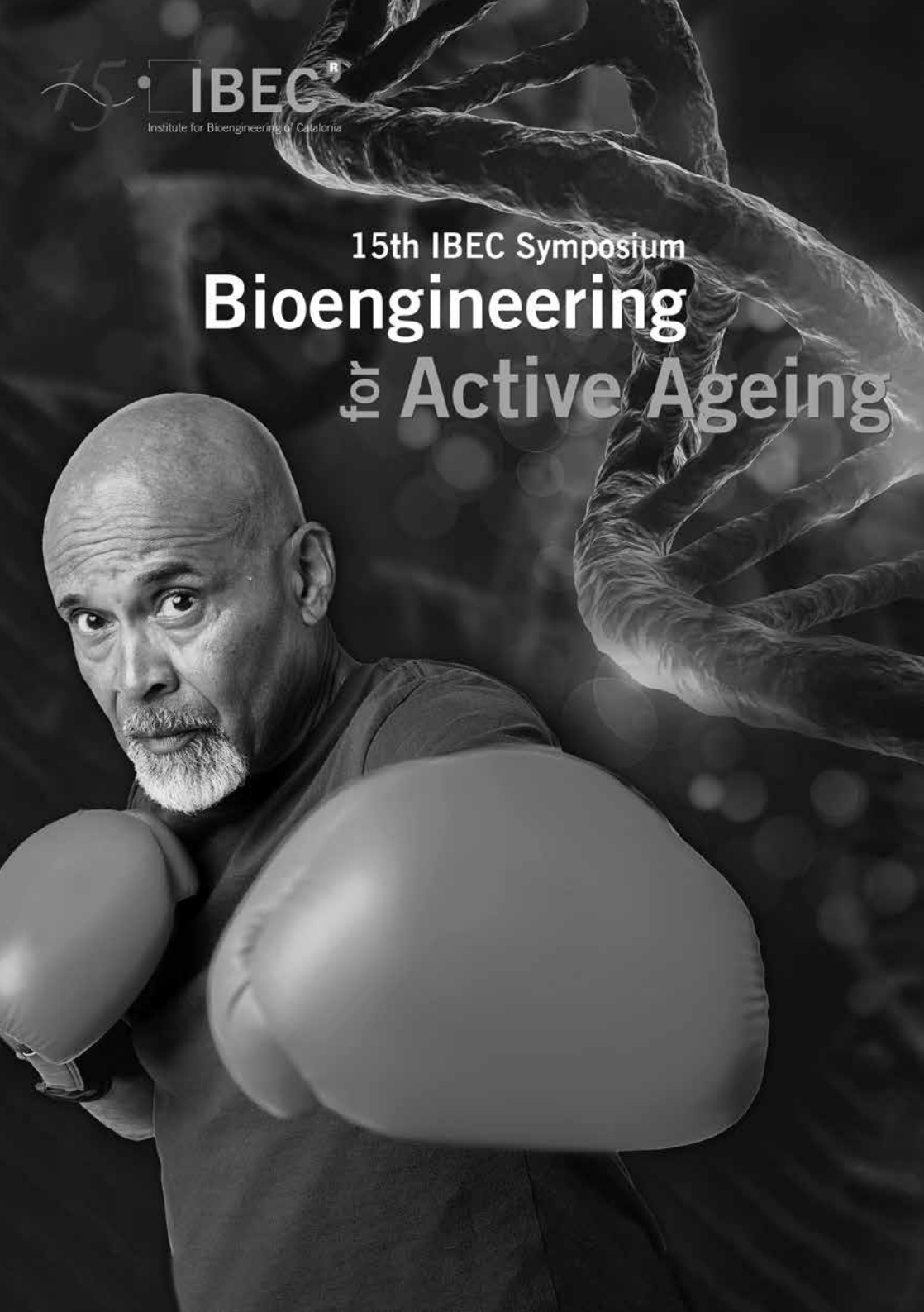
15 IBEC
Institute for Bioengineering of Catalonia
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EXCELENCIA SEVERO OCHOA

Welcome to IBEC's 15th 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 Active ageing. I hope that you'll be stimulated and inspired by our programme of talks, posters, and networking.

This year, we have many reasons to celebrate. It is the 15th anniversay of IBEC and after two years of meeting oline we will be meeting all back face to face. This will be an opportunity to share our science, make new connections and also reconnect.

Thank you very much for participating in the Symposium!

Josep Samitier

Director
Institute for Bioengineering of Catalonia (IBEC)

15th IBEC Symposium
Bioengineering for Active Ageing

Programme

Wednesday 19th October

08:30 – 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	Keynote speaker How to activate drugs, proteins and genes by ultrasound employing principles of mechanochemistry **Andreas Hermann**, Leibniz Institute for Interactive Materials · Aachen University, Germany *Chair: César Rodriguez-Emmenegger*
10:20 – 11:00	Coffee break
11:00 – 11:45	Flash poster presentation · Session I *Chair: César Rodriguez-Emmenegger and Elena Garreta*
11:45 – 12:20	Keynote speaker Targeting Protein Aggregation in Neurodegenerative Diseases **Michele Vendruscolo**, University of Cambridge *Chair: Giuseppe Battaglia*
12:20 – 12:50	Technology Transfer session **Laia Arnal Arasa**, Business development director, Vall d'Hebron Institute of Research (VHIR) **Damià Tormo**, Columbus Venture Partners *Chair: Eduardo Salas*
13:00 – 14:30	Lunch

14:30 – 15:25	Flash poster presentation · Session II *Chair: Iris Batalha and Jordi Alcaraz*
15:25 – 16:00	Keynote speaker Low-modulus bone cement - from vertebroplasty to discoplasty **Cecilia Persson**, Uppsala University *Chair: Samuel Sánchez*
16:00 – 16:20	Alumni session A Journey through Emerging Scaffold-based Strategies for Neural Regeneration **Zaida Álvarez**, Institute for Bioengineering of Catalonia (IBEC) *Chair: Elisabeth Engel*
16:20 – 17:20	Poster session
17:20 – 17:55	Keynote speaker Integrating *in vivo* and *in vitro* data provides insights into dynamic regulation of human epidermal differentiation **Fiona M. Watt**, EMBL Heidelberg *Chair: Josep Samitier*
17:55 – 18:10	Time for PhD Committee Miquel Bosch
18:10 – 18:20	Time for Postdoc Committee Denitza Denkova, Aurora Dols, Juanma Fernández
18:20 – 18:30	Awards and closing ceremony


IBEC

Keynote Lectures

KEYNOTE LECTURE

How to activate drugs, proteins and genes by ultrasound employing principles of mechanochemistry

Andreas Herrmann

DWI – Leibniz Institute for Interactive Materials, Aachen, Germany
RWTH Aachen University, Aachen, Germany

The field of optogenetics has enabled the fundamental understanding of neural circuits and disorders.[1,2] However, current optogenetic techniques require invasive surgical procedures to deliver light to target cells due to the low penetration depth of light into tissue. Therefore, ultrasound (US) was used as alternative trigger since US can deeply penetrate tissue with high spatiotemporal control. Our group develops general molecular technologies based on nucleic acid aptamers and mechanochemistry to control the activity of proteins and drugs (Fig. 1) by US.[3,4] Therefore, we produce high molecular weight polynucleic acids by rolling circle amplification or transcription that encode multiple aptamer binding sites for proteins or drugs. Once these loaded nucleic acid carriers are subjected to ultrasonication, covalent and non-covalent bond cleavage occurs by collapse of US-induced cavitation bubbles leading to activation of protein or drug cargoes. Similarly, we liberate small bioactive trigger molecules by US that initiate gene expression involving riboswitches relying on modified tRNA scaffolds. [5] A particular emphasis is paid to reducing US energies to make these sonogenetic and sonopharmacological systems compatible with living matter.[3]

Keywords: polymer-mechanochemistry, sonogenetics, sonopharmacology, drug delivery

References:

[1] W. Haubensak, P. S. Kunwar, H. Cai, S. Ciocchi, N. R. Wall, R. Ponnusamy, J. Biag, H.-W. Dong, K. Deisseroth, E. M. Callaway, M. S. Fanselow, A. Lüthi, A. J. Anderson, Nature 468, 270 (2010).

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[5] A. Paul, E. M. Warszawik, M. Loznik, A. J. Boersma, A. Herrmann, Angew. Chem. Int. Ed. 59, 20328 (2020).

Andreas Herrmann

Andreas Herrmann studied chemistry at the University of Mainz (Germany). From 1997 to 2000 he pursued his graduate studies at the Max Planck Institute for Polymer Research in Mainz in the group of Prof. Klaus Müllen. Then he worked as a consultant for Roland Berger Management Consultants in Munich (2001). In the years 2002 and 2003 he returned to academia as a postdoctoral fellow working on protein engineering with Prof. Don Hilvert at the Swiss Federal Institute of Technology, Zurich. In 2004 he was appointed as a head of a junior research group at the Max Planck Institute for Polymer Research.

From 2007 to 2017 he held a position as full professor at the Zernike Institute for Advanced Materials at the University of Groningen, The Netherlands, where he headed the chair for Polymer Chemistry and Bioengineering.

Since June 2017 he is scientific board member of the DWI – Leibniz Institute for Interactive Materials in Aachen, Germany, and fills a position as full professor at RWTH Aachen University for Macromolecular Materials and Systems. In 2018, he became vice-director of the DWI – Leibniz Institute.

KEYNOTE LECTURE

Targeting Protein Aggregation in Neurodegenerative Diseases

Michele Vendruscolo

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

The process of protein aggregation is linked to a wide range of human disorders that include Alzheimer's and Parkinson's diseases. The oligomeric intermediates produced during this process are increasingly recognized as highly cytotoxic. 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 describe a kinetics-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 this strategy for the amyloid beta peptide, which is closely associated with Alzheimer's disease. As this strategy is general, it can be applied to oligomers of other proteins in drug discovery programmes.

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.

Laia Arnal Arasa
Business Development Director
Vall d'Hebron Institute of Research (VHIR)

Laia Arnal is biologist (University of Barcelona) with an Executive MBA, and Advanced Management Programme in Health Institutions by IESE Business School. Laia has +15 experience in fostering valorization and technology transfer and business development in biotechnology and biomedicine. She's been +8 years in the promotion and dinamization of the biotechnology, biomedical and medical technologies cluster in Catalonia, Spain in BIOCAT BioRegió de Catalunya, fostering strategic initiatives like the European Institute of Technology Kick- EITHealth and several programs of entrepreneurship in life sciences (BioEmprenedorXXI; MOEBIO d·HEALTH) and has been director of the International Center for Scientific Debate (B·DEBATE).

Since 2016 she is the Business Development Director at VHIR. Responsible for the implementation of the Business Development Strategy, and in charge of the technology transfer and Innovation Unit, fostering licenses, spin-offs, start-ups. She participates in the Identification, negotiation and consolidation of new business opportunities at national and international level. Establishment of strategic alliances. Fosters disruptive and collaborative R+D+I biomedical projects throughout the Vall d'Hebron University Hospital Campus. She is member of the Steering Committee of ITEMAS, Platform of the Carlos III Health Institute (ISCIII) to support R&D&I in Biomedicine and Health Sciences, and co-coordinator of the European University Hospital Alliance (EUHA) Innovation Network.

Damià Tormo
Managing Partner and co-founder
Columbus Venture Partners

Damià Tormo is Managing Partner and co-founder of Columbus Venture Partners. He has spent the last 20 years working in the biotech industry and has founded several biotech companies including Highlight Therapeutics, Artax Biopharma, Viralgen (adquired by Bayer), Vivet Therapeutics (option by Pfizer), PTS (Adquired by Arcline), Tyris Therapeutics, Recovid (adquired by Vitro) or Radioterapia de Protones.

Damia is currently Director of Artax, Highlight, Tyris, Minoryx, Aleta, Integra and Syngoi. He has also served on the Boards of Sanifit (adquired by Vifor), Algenex (adquired by Insud), Recovid, Viralgen and PTS.

Moreover, Damia is the President of Fundación Columbus, a non-profit organization that develops advanced therapies for children with cancer and ultra-rare genetic diseases.

He holds a PhD in in Immunology and Molecular Genetics from the Rheinische Friedrich-Wilhelms-Universität Bonn and has been researcher at Uniklinik Bonn, University of Michigan or the Spanish National Cancer Center (CNIO)

KEYNOTE LECTURE

Low-modulus bone cement - from vertebroplasty to discoplasty

Celia Persson

Uppsala University
Competence Centre in Additive Manufacturing for the Life Sciences

Low-modulus bone cements have been developed in an attempt to mitigate complications associated with vertebroplasty. Many fractures occurring after treatment are believed to be avoidable, through the use of less cement and/or a low-modulus cement, with properties better suited to the surrounding bone. Discoplasty, i.e. injection of cement into degenerated discs presenting vacuum phenomena, has recently been returned to as a possible solution for elderly patients suffering from comorbidities, as it presents a less invasive alternative.

Here as well, a low-modulus cement may be beneficial. We have developed a low-modulus cement, currently under clinical evaluation for vertebroplasty. However, its potential benefits in discoplasty remain to be determined. Very few pre-clinical models are available to this end, and here I will report on results from a computational study, as well as the development of an experimental ovine model for future use in discoplasty evaluations. The translational process of the cement will also be commented upon.

Celia Persson

Cecilia Persson is Head of the Division of Biomedical Engineering at Uppsala University (https://katalog.uu.se/profile/?id=N9-1332) and the President of the Scandinavian Society of Biomaterials. She obtained her PhD in mechanical engineering from the Institute of Medical and Biological Engineering (iMBE) in 2009.

Further, she received the Göran Gustafsson Foundation Small Prize to Your Researchers in 2014, appeared in the 100-list of the Royal Swedish Academy of Engineering Science with AM4Life in 2022 and she was awarded with the Lilly and Sven Thuréus Prize of the Royal Society of Sciences at Uppsala in 2022.

She currently directs research networks within the field of biomaterials and devices for the spine (MSCA ITN NU-SPINE and EIT Health SOFTBONE), as well as a Competence Centre for Additive Manufacturing in the Life Sciences (https://www.uu.se/en/research/am4life/). She is the author of 114 peer-reviewed international publications with more than 2600 citations and a H-index of 29.

A Journey through Emerging Scaffold-based Strategies for Neural Regeneration

Zaida Álvarez

Institute for Bioengineering of Catalonia (IBEC)

One of the challenges facing the neuroscience field is the development of effective therapies that can enhance the regenerative capacity of the central nervous system based on the advances achieved in basic research. During the last decades, biomaterials have continuously been tested as central players for a wide range of CNS regenerative strategies, particularly the development of highly biocompatible 3D tissue-engineered scaffolds proficient to bridge the lesion site.

During my career at IBEC and Northwestern University, I have been focused on the design and implementation of different biomaterials approaches to target different aspects of the regenerative response. In this talk, I will focus on my scientific trajectory and particular emphasis will be given to my latest research on dynamic supramolecular peptide amphiphiles for spinal cord injury regeneration and disease

Zaida Álvarez

Zaida Alvarez is currently a Ramon y Cajal and leading investigator of the biomaterials for neural regeneration group at the Institute for Bioengineering of Catalonia (IBEC), Spain. She earned her PhD degree in Biomedical Engineering with Prof. Elisabeth Engel at Polytechnic University of Catalonia in 2014. In 2015, as a self-funded postdoc she joined Professor Samuel Stupp's laboratory, at Northwestern University in Chicago to work on peptide amphiphiles for neural regeneration where she published more than 15 papers in high impact factor journals such as Science, Nature nanotechnology, nature communications, Advanced Science or Nanoletters.

In 2019, she was promoted as an assistant professor at the department of medicine, at Feinberg Medical school at Northwestern University where she continued her research in spinal cord regeneration and in vitro platforms for iPs modelling.

She is also consulting engineering in a couple of companies in the USA, she has 4 patents already transferred to AmphixBio Incorporation and got numerous awards such as Young Baxter investigator award in 2019, and Rafael Hervada award in 2021

KEYNOTE LECTURE

Integrating *in vivo* and *in vitro* data provides insights into dynamic regulation of human epidermal differentiation

Fiona M. Watt

EMBL Heidelberg

The interfollicular epidermis is the multilayered epithelium that forms the outer layer of the skin. It is maintained by stem cells that are attached to a basement membrane. Cells undergo terminal differentiation as they detach from the basement membrane and move through the suprabasal epidermal layers to the tissue surface, from which they are shed.

While many of the molecular regulators of stem cell behaviour have been identified, how they are integrated and change over time are open questions. I will describe how new insights from single cell RNA sequencing of cells isolated directly from the skin, combined with new experimental models, are helping us to understand the combined effects of cell-ECM adhesion and cell-cell contact in regulating epidermal differentiation

Fiona M. Watt

Fiona Watt obtained her first degree from Cambridge University and her DPhil, in cell biology, from the University of Oxford. She was a postdoc at MIT, where she first began studying differentiation and tissue organisation in mammalian epidermis. She established her first research group at the Kennedy Institute for Rheumatology in London and then spent 20 years at the CRUK London Research Institute. She helped to establish the CRUK Cambridge Research Institute and the Wellcome Trust Centre for Stem Cell Research and in 2012 she moved to King's College London to set up the Centre for Stem Cells and Regenerative Medicine. From 2018 to 2022 she was on secondment as Executive Chair of the Medical Research Council. In 2022 she moved to Heidelberg where she runs a lab at EMBL and is EMBO Director

FLASH presentations

FLASH PRESENTATIONS · SESSION 1

NANOMEDICINE

NAME	SURNAME	TITLE
Silvia	Acosta Gutierrez	The role of the cell glycocalyx on nanoparticle's binding
Marc	Azagra	High-throughput dynamic nuclear polarization magnetic resonance imaging (DNP-MRI) analysis in a microfluidic multiwell device
Barbara	Borges Fernandes	The minimal chemotactic cell
Juan	Fraire	Light-Triggered Mechanical Disruption of Extracellular Barriers by Troops of Enzyme-Powered Nanomotors for Enhanced Delivery
Sujey	Palma Florez	Permeability study of nanotherapeutics agents against neurodegenerative diseases through BBB-on-a-chip
Alba	Rubio Canalejas	Regulation of ribonucleotide reductases in Pseudomonas aeruginosa under oxidative stress conditions
Mireia	Seuma	Using Deep Mutational Scanning to map amyloid nucleation

FLASH PRESENTATIONS · SESSION 2

CELL ENGINEERING

NAME	SURNAME	TITLE
Barbara	Blanco Fernandez	Bioengineering breast tumors with decellularized porcine mammary glands bioinks
Juanma	Fernandez-Costa	Modeling Duchenne muscular dystrophy fibro-adipogenic processes *in vitro* using 3D skeletal muscle co-cultures
Maria	Gallo	Generation Of Reporter Human Pluripotent Stem Cell Lines To Study Cardiac Development And Disease
Elena	Garreta	Engineering cell microenvironments with kidney decellularized extracellular matrix hydrogels to generate kidney organoid models with enhanced vascularization

ICT FOR HEALTH

NAME	SURNAME	TITLE
Dolores	Blanco-Almazán	Estimation of breathing pattern parameters using wearable bioimpedance during walking
Celia	Mallafre Muro	Breath analysis for the detection of pseudomonas aeruginosa infections in bronchiectasis patients using electronic nose and gas chromatography-mass spectrometry

MECHANOBIOLOGY

NAME	SURNAME	TITLE
Amy	Beedle	Fibrillar adhesions provide mechanical memory to the nucleus through the vimentin cytoskeleton
Giulia	Fornabaio	Mechanisms of biogenesis of a novel tumoral intermediate in colorectal carcinomas

FLASH PRESENTATION | NANOMEDICINE

FLASH presented by:

NAME: Silvia Acosta Gutierrez
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

The role of the cell glycocalyx on nanoparticle's binding

Silvia Acosta[1], Giuseppe Battaglia[1,2,3]
[1] Institute for Bioengineering of Catalonia (IBEC) - Barcelona Institute of Science and Technology (BIST)
[2] Catalan Institution for Research and Advanced Studies -ICREA
[3] Department of Chemistry - University College London (UCL)

Significant design efforts are devoted to cell population targeting by nanomedicines in terms of ligands and surface receptors. Still, the cell barrier in health and disease, the glycocalyx, is often neglected. The glycocalyx is the first defence line of the cell, a ~20-30nm complex polysaccharide matrix comprising proteins and complex sugar chains (proteoglycans and glycoproteins). Changes in glycosylation modulate the inflammatory response, enable viral immune escape and promote cancer cell metastasis by altering the physical environment within which the cell-surface receptors (targets) operate. Chemical affinities, chemical rates, molecular transport, and equilibrium configurations at the cell surface depend broadly on the glycocalyx's precise physical structure and charge distribution.

Using a multiscale approach, we have derived a predictive model for nanomedicines-cell association. Our model explicitly considers the nanomedicine interaction with the cell glycocalyx, shedding light on the effect of this barrier on nanomedicine binding and viral attachment to the cell. Our experimental validation of the model shows that the nanomedicine/viral-cell binding energy is a highly non-linear function. Our model enables the rational design of phenotypic nanomedicines, allowing us to tune their cell avidity by varying its radius, degree of polymerisation and number of ligands.

FLASH presented by:

NAME: Marc Azagra
GROUP: Molecular Imaging for Precision Medicine
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

High-throughput dynamic nuclear polarization magnetic resonance imaging (DNP-MRI) analysis in a microfluidic multiwell device

Marc Azagra[1†], J Yeste [1†‡], Maria A. Ortega[1§], Alejandro Portela, Gergő Matajsz[1], Alba Herrero-Gómez[1], Yaewon Kim[2], Renuka Sriram[2], Xiao Ji[2], John Kurhanewicz[2,3], Daniel B. Vigneron[2,3], Irene Marco-Rius[1]*

[1] Institute for Bioengineering of Catalonia, The Barcelona Institute of Science and Technology, Barcelona, Spain.

[2] Department of Radiology and Biomedical Imaging, University of California San Francisco, San Francisco, California

[3] Graduate program in Bioengineering, University of California, Berkeley and University of California, San Francisco, California, USA

[†] Authors contributed equally to this work

Dynamic nuclear polarization (DNP)-nuclear magnetic resonance (NMR) technique enhances the intrinsically low sensitivity of NMR up to 10,000 times. This high sensitivity makes this technique very attractive for real-time in either in-situ or in-vivo metabolic analysis. While the costly and time-consuming hyperpolarization of the metabolites hinder its broad application, its low throughput has also shown to be insufficient to provide repeatability; DNP-NMR experiments take hours to prepare and rely on the rapid decay (~30–60 s) of nuclear spins polarization. To overcome this limited production, we have successfully increased experimental throughput by developing a microfluidic DNP-magnetic resonance spectroscopic imaging (MRSI)-based method for real-time metabolic studies; experiments with up to 8 replicates can be performed by one single DNP shot. Thus, relevant amount of data can be efficiently generated. Combined DNP-MRSI and microfluidics has allowed us to create a repeatable method for tracing fast reaction kinetics. Here, we demonstrate the application of the method, tracking the oxidation reaction of hyperpolarized [1-13C]pyruvic acid with hydrogen peroxide testing 2 different conditions as a proof of concept. The proposed method represents a new high-throughput approach for hyperpolarization-enhance NMR experimentation that, ultimately, led to preclinical tools in which analytical techniques can be applied non-invasively in situ for diseases that affect cellular metabolism.

FLASH PRESENTATION | NANOMEDICINE

FLASH presented by:

NAME: Barbara Borges Fernandes
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

The minimal chemotactic cell

Bárbara Borges Fernandes[1,2], Azzurra Apriceno[1], Safa Almadhi[3], Ian Williams[5] and Giuseppe Battaglia[1,3,4]
[1]Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain
[2]Department of Physics, University of Barcelona, Barcelona, Spain
[3]Department of Chemistry, University College London, London, UK
[4]Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain
[5]Department of Physics, University of Surrey, Guildford, UK

Cells and microorganisms like bacteria use chemotaxis to move in a directed manner in response to concentration gradients of nutrients and toxins. Likewise, synthetic delivery systems could take inspiration from nature to mimic this kind of transport. Ultimately, it could guide nanomotors in the body in a specific way, according to concentration gradients occurring physiologically. We present a system with the basic characteristics to achieve a minimal chemotactic cell. It consists of an asymmetric phospholipid vesicle of 100 nm (liposome) with an encapsulated enzyme (glucose oxidase). The asymmetry is given by the presence of pores in the membrane, inserted by the protein alpha-hemolysin. Mass ratios of 0.075 and 0.1 Hly/lipid were used, resulting in ~2 and ~3 pores per liposome. When the liposome is placed in an environment with glucose, the catalysed reaction occurs in its lumen. The products diffuse outwards through the pores, creating a local concentration gradient.

The asymmetric distribution of products along its surface generates a slip velocity that moves the vesicle in response to the glucose concentration gradient. The active motion of the liposomes labelled with rhodamine octadecyl ester perchlorate (1%) was investigated in an Ibidi microfluidic device in which a concentration gradient of 0.05 M of glucose was established in the channel. While the pristine liposome and the one with encapsulated glucose oxidase (GOX-L) presented a velocity towards low glucose concentration, the displacement of liposomes with pores (~3) was reverted to the opposite direction. The movement of the pristine liposome and the GOX-L are due to diffusioosmophoresis, as a result of the interaction of glucose and the channel walls. In the absence of a glucose concentration gradient, vesicles present only Brownian motion. The drift velocity is the sum of the diffusioosmophoresis velocity and the chemotactic velocity. With ~2 pores, the chemotactic velocity is on the same order magnitude of the diffusioosmophoresis, cancelling out any drift movement. With ~3 pores, the chemotactic velocity suppresses the diffusioosmophoresis, resulting in a net drift with a speed of ~-0.7 microm/s towards high glucose concentrations.

FLASH presented by:

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

Light-Triggered Mechanical Disruption of Extracellular Barriers by Troops of Enzyme-Powered Nanomotors for Enhanced Delivery

[1]*Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST)*

[2]*Laboratory for General Biochemistry and Physical Pharmacy, Faculty of Pharmaceutical Sciences, Ghent University*

Nanomotors (NMs) has been suggested as the new generation of carriers in drug delivery. Despite their promising capabilities for tissue penetration and accumulation, their delivery efficiency is hindered by the different physical extracellular barriers, for which active motion solely do not guarantee successful trespassing. Here we explore the use of enzyme-powered NMs with the novel capability of inducing light-triggered formation of vapor nanobubbles (VNBs), that allow to generate fluid phenomena with mechanical disruptive effects. We show that these motors, named the 1st troop, can collectively displace towards collagen type 1 protein fibers acting as barrier model, accumulate onto and completely disrupt them upon irradiation. Microenvironment modification induced by these NMs (1st troop) were evaluated by quantification of the delivery efficiency of a second type of fluorescent NMs (2nd troop) in HeLa cells pre-cultured inside phantom microfluidic channels. In the absence of collagen fibers, 2nd troop depict a 12-fold increase in the delivery efficiency in comparison to passive particles (absence of fuel).

In the presence of the collagen fibers blocking the microfluidic channel, successful intracellular delivery of the 2nd troop occurs only after sequential treatment and irradiation with the 1st troop, allowing a 10-fold increase in delivery efficiency when compared with the non-irradiated condition. The improvement in delivery efficiency achieved by sequential treatment with NM troops that present collective displacement, combined with the capability to induce mechanical disruption of biological barriers, could pave the way toward novel therapies for trespassing these barriers and enhance delivery of drug-loaded NMs.

FLASH PRESENTATION | NANOMEDICINE

FLASH presented by:

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

Permeability study of nanotherapeutics agents against neurodegenerative diseases through BBB-on-a-chip

Sujey Palma-Florez[1,3], Marta Perxes[9], Daniel Gonzalez Carter[4], Santiago Grijalvo[2,8], Adrián Lopez[3,5], Francisco Moralez-Zavala[6,7], Sara Gorberna[9], Oscar Castaño[5,3], Pedro Gomez[9], Marcelo Kogan[6,7], Josep Samitier[2,1,3], Mónica Mir[2,1,3], Anna Lagunas[2,1]

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In recent years, neurodegenerative diseases (NDDs) have become more prevalent because life expectancy has increased. NDDs imply a detriment in the quality life because neurodegenerative process is associated with memory loss, cognition impairment and behavioral alterations. Circumstances such as the production of reactive oxygen species (ROS) or extracellular accumulation of beta amyloids aggregates are some of hallmarks in Alzheimer's diseases. To combat this phenomenon, nanotechnology proposes several alternatives as treatment for NDDs and it is necessary to evaluate them in reproducible, cheaper, and animal-free models as organ-on-a-chip (OoC). Recently, several devices have been developed to mimic biological barriers in the brain such as the blood-brain barrier (BBB) to evaluate drug efficacy and disease progression. In addition, detection platforms can be incorporated in BBB-on-a-chip to monitor the barrier integrity through trans-endothelial electrical resistance (TEER). In this work, we present the development of a BBB-oC model with an integrated TEER-monitoring system composed by a 3D co-culture of human endothelial, astrocytes and pericytes cells to assess the drug permeability of several nanoparticles. We evaluated the permeability of polymeric nanoparticles with antioxidant scavenging radical agents through fluorescent time-lapse imaging. Also, we analysed the permeability of nanosystems that avoid the beta amyloid aggregates formation and evaluate the influence of BBB shuttle peptides to entrance in the brain. Finally, this platform could be useful to elucidate the influence of nanoparticles-endothelium interaction in brain by immunofluorescence and TEER measuring. In conclusion, our BBB-oC system could be a useful tool to assess the permeability of promising nanosystems in a cheaper and high-throughput manner for new treatment discovery against NDDs.

FLASH presented by:

NAME: Alba Rubio Canalejas
GROUP: Bacterial infections: antimicrobial therapies
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Regulation of ribonucleotide reductases in Pseudomonas aeruginosa under oxidative stress conditions

**Alba Rubio-Canalejas[1], Joana Admella[1], Lucas Pedraz[1], Eduard Torrents[1,2,*]*

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Ribonucleotide reductases (RNR) are key enzymes that catalyze the synthesis of deoxyribonucleotides, the monomers needed for DNA replication and repair. RNR are classified into three classes (I, II, and III) depending on their overall structure and metal cofactor. Organisms whose genome encodes for several RNR classes are remarkably adaptable to different environments. Pseudomonas aeruginosa is an opportunistic pathogen harboring all three classes of RNR, increasing its metabolic versatility. It is known that during an infection, P. aeruginosa can grow to form a biofilm to be protected from the host immune defenses, such as the production of reactive oxygen species by macrophages. One of the essential transcription factors needed to regulate biofilm growth and other metabolic pathways is AlgR. AlgR is part of a two-component system where FimS is a kinase that catalyzes its phosphorylation in response to external signals. Additionally, AlgR is part of the regulatory network of the cell RNR regulation. In this study, we delved into the regulation of the RNR through AlgR under oxidative stress conditions. We have determined that the non-phosphorylated form of AlgR is responsible for the class I and II RNR induction after H2O2 addition in planktonic culture and during flow biofilm growth. We observed a similar RNR induction pattern comparing the P. aeruginosa laboratory strain PAO1 with different P. aeruginosa clinical isolates. And finally, we showed that during a Galleria mellonella infection, where oxidative stress is highly produced, AlgR was crucial to induce class II RNR (nrdJ). Thereby, we showed that the non-phosphorylated form of AlgR, besides being crucial for infection chronicity, regulates the RNR network in response to oxidative stress during infection and biofilm formation.

This study was partially supported by grants RTI2018-098573-B-I00 and PID2021-125801OB-100 funded by MCIN/AEI/10.13039/501100011033 and "ERDF A way of making Europe", the CERCA programme and AGAUR-Generalitat de Catalunya (2017SGR-1079), the European Regional Development Fund (FEDER), Catalan Cystic Fibrosis association and Obra Social "La Caixa". A.R.C. got a grant (PRE2018-083709) funded by MCIN/AEI/10.13039/501100011033 and "ESF Investing in your future".

FLASH PRESENTATION | NANOMEDICINE

FLASH presented by:

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

Using Deep Mutational Scanning to map amyloid nucleation

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Protein assemblies in the form of insoluble amyloids are associated with many different human diseases, but how mutations impact protein aggregation is not well understood. There has been no *in vivo* large-scale analysis of the effects of mutations on amyloid formation, and this has limited our understanding of proteins that are able to inhabit different physical states and that, for example, can transition from liquid condensates to solid aggregates.

To address this shortcoming, I developed a Deep Mutational Scanning (DMS) method to report on the aggregation of thousands of protein sequences in parallel. I applied this systematic approach to the amyloid beta (Aß) peptide, as a model of classical amyloids. Self-assembly of Aß into amyloid fibrils is a hallmark of Alzheime's disease (AD) and specific dominant mutations in Aß also cause rare familial forms of AD (fAD). However, the fAD known mutations only represent a tiny fraction of all possible mutations in Aß and it is not clear how they alter its aggregation. By quantifying *in vivo* amyloid fibril nucleation for >14,000 variants of Aß, I generated the first comprehensive map of how mutations alter the nucleation of amyloids by any protein. Our results reveal a modular organization of mutational effects along the Aß sequence. They also uncover the role of charge and specific gatekeeper residues in the disordered N-terminus in preventing nucleation. Strikingly, the *in vivo* nucleation scores, unlike computational predictors and previous measurements, accurately discriminate all known fAD mutations. This suggests that accelerated nucleation is the fundamental molecular mechanism by which mutations cause fAD. Taken together, these results provide the first global picture of how sequence changes prevent and promote the nucleation of amyloid fibrils and provide a clinically-validated resource for the future interpretation of mutational impact in Aß, opening a new path for personalized therapy in AD. Moreover, our work illustrates the power of DMS in illuminating sequence-to-activity relationships and we suggest that this approach should be used to target other protein sequences that undergo a process of self-assembly upon mutation, including those disordered domains that transition from a liquid de-mixed state to insoluble fibrils.

FLASH presented by:

NAME: Barbara Blanco Fernandez
GROUP: Biomaterials for regenerative therapies
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Bioengineering breast tumors with decellularized porcine mammary glands bioinks

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Breast cancer is the most common cancer in women worldwide. Pharmaceutical Industry invests a great deal of resources in finding new treatments, but most of them fail in clinical trials, evidencing that current preclinical models cannot mimic human tumors. The breast tumor microenvironment (TME) is formed by cancer cells (BCCs), stromal cells, extracellular matrix (ECM) and soluble factors, being all involved in tumor progression and drug response. Bioprinted 3D *in vitro* models have emerged as promising platforms for understanding cancer progression recapitulating the tumor organization.1 In addition, the importance of the ECM in tumor progression has motivated the synthesis of more biomimetic bioinks. Among them, decellularized tissues-derived matrices (TDMs) can provide the native biological cues, but its inadequate mechanical properties prevent their bioprinting. The aim of this work is to develop a breast TDM-derived bioink suitable for bioprinting breast cancer models.

Porcine mammary glands were decellularized, and its composition was evaluated. Cell-laden bioinks were prepared by combining TDM digested with pepsin, rheological modifiers and BCCs. Scaffolds were bioprinted with a bioplotter (RegenHU) and crosslinked. Bioinks were further tuned by adding collagen I (Col1) which is overexpressed in breast tumors. Bioinks' printability and mechanical properties were determined. The cellular survival and proliferation; morphology; e-cadherine and multidrug resistance and malignancy gene expression, and doxorubicin efficacy were studied. Col1 gels were used as controls. Models including mesenchymal stem cells were also bioprinted.

Mammary glands were efficiently decellularized, having a high content in collagen and glycosaminoglycans. Bioinks of TDM alone could not be bioprinted, and it was necessary to add gelatin methacrylamide and alginate (TGA). The presence of Col1 into the bioink (TGAC) improved ink printability and shape fidelity.2 TGA and TGAC hydrogels had Young modulus of ≈4kPa, recapitulating tumors stiffness. BCCs had a good viability up to 14 days, forming spheroids with low e-cadherin expression. The Col1 presence in the bioink increase in BCCs proliferation but did not increase their malignancy and drug resistance. Indeed, TGA showed an upregulation of fibronectin and multidrug resistance protein 1 and higher drug resistance due to an upregulation of HSP90AB1 in comparison to Col1 and TGAC, suggesting a higher malignancy of BCCs in the absence of Col1.2. TDM bioinks closely recreate the tumor ECM and allow the bioprinting of cancer models with a close recapitulation of the BCCs malignancy.

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FLASH PRESENTATION | CELL ENGINEERING

FLASH presented by:

NAME: Juanma Fernandez-Costa
GROUP: Biosensors for bioengineering
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Modeling Duchenne muscular dystrophy fibro-adipogenic processes *in vitro* using 3D skeletal muscle co-cultures

Juan M. Fernández-Costa[1], Ainoa Tejedera-Villafranca[1], Xiomara Fernández-Garibay[1], and Javier Ramon-Azcon[1,2]

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Duchenne muscular dystrophy (DMD) is a devastating genetic myopathy that affects 1 in 3,500 male births and has no cure to date. It is caused by mutations in the dystrophin gene. Myonecrosis, sarcolemmal disorganization, inflammation and accumulation of fibro-fatty scar tissue appear early in DMD and increase with age. Patients develop muscle weakness early in life that progresses over time and leads to loss of ambulation during adolescence and respiratory and cardiac complications leading to death before age 30. The process of muscle degeneration involves a complex interplay between multiple cell lineages spatially localized within damaged areas, termed the degenerative niche, including inflammatory cells, satellite cells (SCs) and fibroadipogenic precursor cells (FAPs). Understanding the molecular pathways that drive fibro-adipogenic processes in DMD is key to shed light on the molecular pathogenesis of the disease and develop new treatments. Here we propose to use 3D skeletal muscle co-culture models to characterize the biological properties of FAPs and how they interact with myotubes. To this end, 3D bioengineered tissues were developed by encapsulating FAPs and myoblasts in a fibrin-composite matrix using a PDMS casting mold. When FAPs are induced to differentiate into fibroblasts or adipocytes, FAPs from DMD patients produce an increase in both collagen I and fat in 3D co-cultures. In addition, the physiological response of the 3D skeletal muscle co-cultures after electrical pulse stimulation is altered by the effect of DMD FAPs on contraction dynamics and the force exerted by the tissues on the PDMS pillars. Both these DMD-related structural and functional phenotypes reproduced in 3D skeletal muscle cocultures will be interrogated to test drug candidate molecules that potentially modulate the fibro-adipogenic process identified in a previous high-throughput drug screening. Interestingly, as the molecular pathways governing muscle degeneration are possibly shared by different muscular dystrophies, the developed research could be used as preliminary data for other muscle diseases facilitating the development of new effective therapies not only for DMD but also for other genetic skeletal muscle disorders such as limb-girdle muscular dystrophies.

FLASH presented by:

NAME: Maria Gallo
GROUP: Pluripotency for organ regeneration
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Generation Of Reporter Human Pluripotent Stem Cell Lines To Study Cardiac Development And Disease.

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The development and use of human pluripotent stem cells (hPSCs) represent an effective tool to recapitulate characteristics related to tissue differentiation, morphogenesis and conversely, human disease. Research exploits the inherent capacity of hPSCs to generate three dimensional (3D) self-organized organ-like structures, the so-called organoids, so that both tissue development and disease can be dissected in the Petri dish. In this context, the advent of CRISPR-Cas9 technology has now allow to incorporate permanent or transient changes in the DNA of living organisms and cells. Our laboratory has developed a cellular platform, named iCRISPR2 (iC2), that allows to perform highly efficient genome editing in hPSCs. In this system, TALEN-mediated gene targeting is used to firstly introduce a doxycycline-inducible Cas9 expression cassette at the safe harbour locus AAVS1 . Cas9 expressing hPSCs offer the possibility to generate reporter, knock-out and knock-in hPSCs. In this work we have exploited this system to generate cardiac reporter cell lines. To this aim we designed highly active and specific sgRNAs which upon nucleofection induceCas9-mediated site-specific double strand breaks (DSB) in the presence of a specific double strand DNA donor sequence that incorporates the sequence of a specific reporter gene. Upon expansion and manual selection of isogenic individual clones we did perform genotyping analysis and identified cardiac reporter cell lines mirroring the endogenous expression of MYH6, MYL2, and SIRPA. Nowadays we are exploiting these lines to define new approaches for the generation of self-assembled cardiac-like organoids which have been characterized at the transcriptomic level by single cell RNA sequencing, conventional molecular biology techniques, and confocal microscopy analysis. Our cardiac-like organoids are nowadays subjected to cell culture conditions emulating early stages of diabetic cardiopathy to establish a platform to interrogate for transcriptional and functional changes during disease.

FLASH PRESENTATION | CELL ENGINEERING

FLASH presented by:

NAME: Elena Garreta
GROUP: Pluripotency for organ regeneration
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Engineering cell microenvironments with kidney decellularized extracellular matrix hydrogels to generate kidney organoid models with enhanced vascularization

Elena Garreta[1], Maria Gallo[1], Daniel Moya-Rull[1], Patricia Prado[1], Carolina Tarantino[1], Andrés Marco[1], Asier Ullate-Agote[2,3,4], Amaia Vilas-Zornoza[2,3,4], Rafael Mestre[5], Samuel Sanchez[5,6], Pedro Aguiar[7], Fritz Dieckman[7], Josep Maria Campistol[8], Felipe Prosper[2,3,4], Nuria Monsterrat[1,6]

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The extracellular matrix (ECM) provides both a physical support and a biochemical microenvironment which are essential during kidney development and homeostasis. However, strategies aiming to modulate cell to ECM interactions during kidney organoid derivation from human pluripotent stem cells (hPSC) remain largely unexplored. Here we present a novel procedure to derive kidney decellularized extracellular matrix (dECM) hydrogels from porcine and human renal cortex as new biomaterials to enrich cell-to-ECM crosstalk during the onset of kidney organoid differentiation. To this end we defined new 2D and 3D culture systems that incorporate the use of kidney dECM hydrogels for kidney organoid generation from hPSC. hPSC were first differentiated towards renal progenitor cells (RPCs) in a process that lasts 4 days. The cellular composition of hPSC-derived RPCs was interrogated using single cell RNA sequencing to further reveal on the signature of ECM and kidney differentiation markers. Next, RPCs were differentiated in the presence of kidney dECM hydrogels for 16 days into kidney organoids which exhibited enhanced differentiation features, including the formation of an enriched endogenous vascular component as determined by immunofluorescence and confocal analysis. To further exploit the potential of this approach to improve the vascularization of kidney organoids, we then derive endothelial-like progenitors from hPSC and established a new procedure to assemble endothelial-like spheroids with kidney organoids in 3D culture to produce vascular-kidney organoids, referred as to vascular-kidney assembloids. Major readouts of kidney and vascular differentiation were studied *in vitro*, and renal cell morphology and vascular network integrity were also evaluated upon implantation of the assembloids into the chick chorioallantoic membrane (CAM) in ovo. Overall, this work shows that exploiting cell-to-ECM interactions during the onset of kidney differentiation allows the establishment of more physiologically relevant kidney organoid models exhibiting enhanced vascularization and maturation, thus increasing the utility of these unique cell culture platforms for drug screening and disease modelling applications.

FLASH presented by:

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

Estimation of breathing pattern parameters using wearable bioimpedance during walking

Dolores Blanco-Almazán[1,2,3], Willemijn Groenendaal[4], Francky Catthoor[5], Raimon Jané[1,2,3]

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Wearable bioimpedance has been proposed to estimate breathing parameters such as respiratory rate (RR). However, its potential application lies in the clinical investigation of daily-life activities like walking. This study evaluated the effect of the walking interference on the detection of respiratory cycles and the estimation of breathing parameters using bioimpedance. 50 chronic obstructive pulmonary disease patients performed static and active measurements during thoracic bioimpedance acquisition. The static measurements included respiratory airflow for reference. The active measurements were used to estimate the walking interference from bioimpedance, and the obtained signals were added to static measurements for comparison with the reference. Afterward, we applied four different preprocessing methods to remove the walking interference and these signals were used to estimate breathing parameters (inspiratory and expiratory times, duty cycle, and RR).

The methods performed differently in terms of accuracy and mean average percentage error (MAPE), showing the need for specific preprocessing for active measurements. Furthermore, the accuracy in respiratory cycle detection was 95 % and the MAPE values in the RR estimation were close to 3 %. The results indicate that breathing parameters can be accurately estimated during walking. Accordingly, the present study reinforces the applicability of wearable bioimpedance for respiratory monitoring in static and active situations.

FLASH PRESENTATION | ICT FOR HEALTH

FLASH presented by:

NAME: Celia Mallafre Muro
GROUP: Signal and information processing for sensing systems
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Breath analysis for the detection of pseudomonas aeruginosa infections in bronchiectasis patients using electronic nose and gas chromatography-mass spectrometry

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[2]*Signal and Information Processing for Sensing Systems Group, Institute for Bioengineering of Catalonia, The Barcelona Institute of Science and Technology, Barcelona, Spain*

Bronchiectasis is a bronchial pathology that can present exacerbation episodes caused by some opportunistic bacteria. In this case, breath samples from patients with stable bronchiectasis and, from patients with exacerbation episodes caused by Pseudomonas Aeruginosa (PA), were collected and analyzed with a GC-MS and an E-Nose. The breath of 13 bronchiectasis patients, 12 bronchiectasis patients suffering a PA infection, and 9 controls was collected into Tedlar Bags. These samples were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) and electronic nose (e-Nose). The e-Nose was a commercial Cyranose 320 with an array of 32 nanocomposite sensors. The data obtained were analyzed with the aim of discriminating the health condition. Chemometric methods were applied, and the obtained results were evaluated with blind samples for the data obtained from the GC-MS. The data obtained from the e-Nose, for improving the gaussianity, was transformed using a non-linear arctangent transformation. K-NN models were built and the classification rates obtained were tested with a double leave one subject out (LOSO) cross-validation. The e-Nose breath analysis was able to separate the 3 groups with a K-NN classification rate of 84% in the 3 classes classification problem. When comparing 2 by 2 classes, the classification rates varied between 84 to 100%, obtaining perfect discrimination between control and bronchiectasis with PA infection samples. These results were tested with external double cross-validation and confirmed by a permutation test. Regarding the GC-MS analysis, the discriminant analysis using PLS-DA reported good results that were not statistically significant in the permutation test. The breath analysis carried with the e-Nose, followed by a strict and proper data analysis, is able to discriminate successfully between control and patients with bronchiectasis and exacerbation episodes. Additionally, GC-MS needs further experiments to increase the number of patients so statistically significant results can be reached.

FLASH presented by:

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

Fibrillar adhesions provide mechanical memory to the nucleus through the vimentin cytoskeleton

Amy EM Beedle[1,2], Aina Albajar-Sigales[1], Joshua Toth[3], Dobryna Zalvidea[1], Zanetta Kechagia[1], Gerhard Wiche[4], Xavier Trepat[1,5], Johanna Ivaska[6], Vivek Shenoy[3], Pere Roca-Cusachs[1,5]

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Forces applied to cell nuclei are increasingly recognized as a major driver of cell function. When forces reach the nucleus, cells require buffering mechanisms to sustain them or dissipate them in a controlled way, but how this occurs is largely unknown. Here we show that the remodelling of fibronectin and the simultaneous formation of fibrillar adhesions locks the nucleus into a mechanically active conformation that is resistant to sudden changes in the mechanical environment of the cell. This encompasses both mechanical deformation and associated signaling (such as for instance via YAP), in response to both a rapid increase or decrease in the mechanical stimuli. Further, we show that the underlying mechanism is the anchoring of the vimentin cytoskeleton to the extracellular matrix through fibrillar adhesions, which maintains nuclear deformation. Our results reveal a new mode of mechanical memory whereby the nucleus is held in place by a long-lived network of fibres, maintaining a mechanically active phenotype long after the mechanical signal is lost.

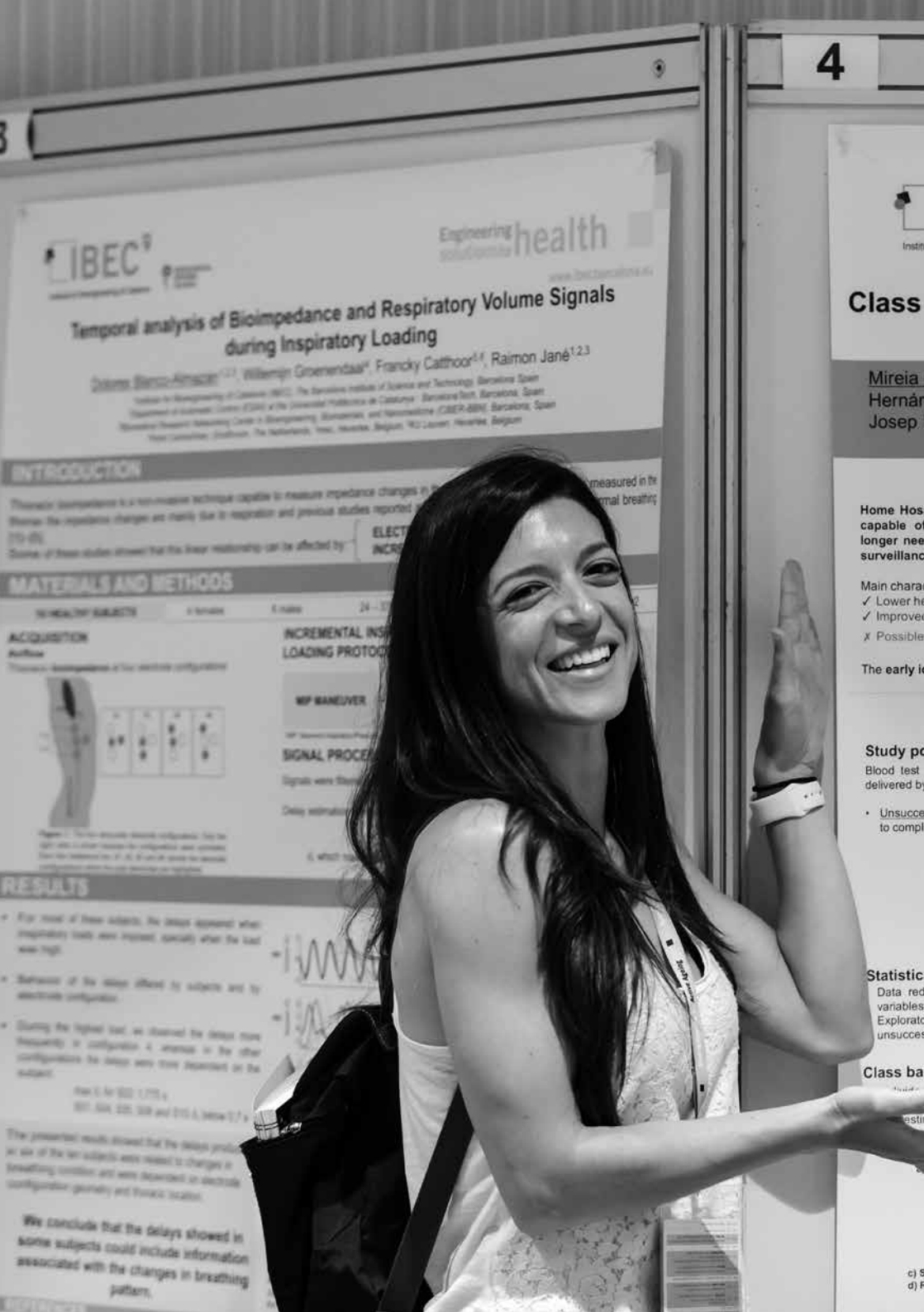
FLASH PRESENTATION | MECHANOBIOLOGY

FLASH presented by:

NAME: Giulia Fornabaio
GROUP: Synthetic Morphogenesis
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Mechanisms of biogenesis of a novel tumoral intermediate in colorectal carcinomas

According to the World Health Organization, cancer is one of the main causes of death worldwide, with colorectal carcinoma (CRC) being the third-leading cause of tumour related-death. The high rate of mortality of CRCs is principally attributed to the metastasis of neoplastic cells from the primary tumour to secondary organs such as the liver, the lung and the peritoneum. These cells can disseminate either as single isolated cells or as collective clusters, undergoing a series of molecular and cellular changes commonly known as Epithelial to Mesenchymal Transition (EMT). However, in 2018, Jaulin's group described a novel modality of peritoneal metastatic spread characterized by the presence of large clusters of cancer cells, which maintain their epithelial properties and display an outward apical polarity. These clusters of cells, termed tumour spheres with inverted polarity (TSIPs), were found in peritoneal effusions of CRCs patients showing early KRAS mutation and hypermethylation of CpG Islands. TSIPs originate through a series of morphological changes: the first event is the sprouting of hypermethylated epithelia, followed by their apical budding, leading to the formation of rounded spherical clusters of cells called buds, and the subsequent cleavage of the newly formed spheres. How cell and tissue mechanics drive this process is still unclear. To provide novel insights into this metastatic cascade, our project aims at deciphering the biomechanical and cellular events regulating the formation of buds in colorectal cancer cell lines. Employing a combination between cellular and molecular biology techniques with biophysical methods, we showed that this process is characterized by over-proliferation and local changes in cell adhesion, coupled with overall MAPK activation. We also demonstrated that buds biogenesis is accompanied by the formation of topological defects, defined as aberrations in cellular orientation, at the level of the monolayer underneath the buds. Interestingly, these topological defects were visible in the epithelium even before bud formation and could therefore be used as predictors. Overall, our study shows that bud development in colorectal cancer epithelia is governed by morphological transitions occurring entirely at multicellular level, rather than by single cells aggregation or cell extrusion. By understanding the formation of these structures, we wish to enlarge our knowledge about collective dissemination in colorectal carcinomas and to identify new potential markers for the progression of this typology of tumour.


4
IBEC
Engineering solutions for health
Temporal analysis of Bioimpedance and Respiratory Volume Signals during Inspiratory Loading
Diana Blanco-Almazan, Willemijn Groenendaal, Francky Catthoor, Ramon Jané
INTRODUCTION
MATERIALS AND METHODS
ACQUISITION
INCREMENTAL INSP
LOADING PROTOC
MIP MANEUVER
SIGNAL PROCES
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 ± 13.0	72.9 ± 14.7	0.072
Male sex, n (%)	1153 (62.3%)	66 (65.3%)	0.633
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 (%).

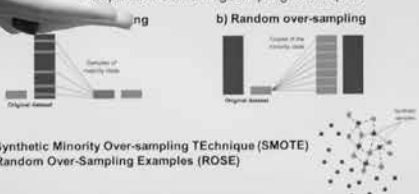
al analysis

lundancy evaluation through **correlation analysis** between pairs of (*Spearman*).
ory 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.

ed compares the following sampling techniques:



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

Results

Correlation analysis

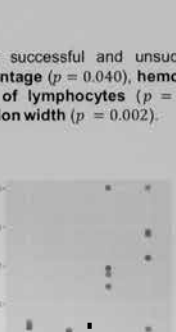
- **Hematocrit** was positively correlated with both **hemoglobin concentration** ($\rho = 0.98$) and **red blood cell count** ($\rho = 0.91$).
- Percentage and total amount of **neutrophils** ($\rho = -0.97$) and **lymphocytes** ($\rho = 0.96$) were highly correlated.

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



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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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POSTER 1 presented by:

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

Differentiation of human pluripotent stem cells into ureteric bud-like cells and evaluation of their ability to recapitulate UB branching morphogenesis in 3D culture

Human pluripotent stem cells (hPSCs), thanks to their unlimited self-renewal capacity and their ability to give rise to cells of all three embryonic germ layers *in vitro* and *in vivo*, represent an ideal tool for the study of human development and disease modelling *in vitro*. Exploiting these inherent properties, hPSCs have been differentiated towards the metanephric mesenchyme (MM) and ureteric bud (UB) lineages, the two progenitor cell populations that reciprocally interact to give rise to the nephrons and the collecting duct of the adult kidney, respectively. Recently, we reported the definition of a methodology to generate hPSC-derived kidney organoids containing nephron-like structures that transcriptionally resembled second-trimester human fetal kidneys. However, as also seen by others, the presence of a proper ramified UB epithelium within the kidney organoids has been not achieved yet. Here, we aim to establish an efficient differentiation methodology to derive UB progenitor cells from hPSC which can be induced to form branching UB-like structures in 3D. To this end we took advantage of a GATA3-mOrange hPSC reporter line developed in our laboratory to screen for the optimal UB induction culture conditions. The percentage of GATA3+ UB-like progenitor cells was assessed by FACS analysis during the time course of differentiation. In addition, the expression of UB markers was analyzed by quantitative PCR (qPCR) and immunofluorescence. Next, we aim to further evaluate the UB identity of the generated cells and their ability to undergo branching morphogenesis by i) aggregating the hPSC-derived UB-like cells with ex vivo mouse embryonic kidney rudiments to reconstitute chimeric kidney organoids, and ii) inducing the formation of hPSC-derived UB-like cell aggregates in the presence of branching inductive signals to generate branching UB organoids. Overall, this approach may lead to expand our knowledge on the early steps defining both MM and UB differentiation and define a unique procedure for the generation of both hPSC-derived UB and MM derivatives that can reciprocally interact in 3D culture giving rise to higher order kidney organoids showing nephron-like structures interconnected by branched ureteric tubular structures.

POSTER 2 presented by:

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

A mutational landscape to understand protein toxicity in neurodegenerative diseases

In the last years, the number of patients suffering from neurodegenerative conditions has massively increased, and their incidence is expected to rise as the population ages. In many of these conditions, the typical pathological hallmarks consist of the formation and deposition of protein aggregates. Here we systematically characterize one of such proteins, Fused in sarcoma (FUS), a DNA and RNA-binding protein that contains a large intrinsically disordered region (IDR) and that is mutated in familial cases of Amyotrophic Lateral Sclerosis (ALS). The mechanism by which these mutations, especially those in the IDR, lead to pathogenesis has not been elucidated yet. To understand how mutations impact FUS toxicity, we used deep mutational scanning (DMS), a high-throughput strategy that allows us to quantify the toxicity of hundreds of mutants. We mutagenised two different FUS domains: the IDR and the RNA recognition motif (RRM), with the aim of understanding if mutations impact in the same way globular (RRM) or disordered regions (IDR). The toxicity landscape of the two regions reveals very different signatures. In the IDR, most mutations are more toxic than wild-type, while in the RRM we uncovered many mutants capable of rescuing FUS toxicity. This toxicity decrease is very position specific since we identified that mutations at residues S300, V301, and G309 have a strong decreasing effect. Taken together, these comprehensive datasets uncover novel features that will help us to systematically elucidate the mechanisms by which FUS becomes toxic upon mutation and to design ways to prevent or rescue such effects.

POSTER 3 presented by:

NAME: Gulsun Bagci
GROUP: Biomaterials for regenerative therapies
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Cell-Derived Extracellular Matrices for Establishment of 3D *in vitro* Tumor Models for Cancer Research

Bioengineered 3D models can recapitulate key properties of the human tumor microenvironment (TME). Indeed, tissue derived, or cell derived extracellular matrix (TDM/CDM) biomaterials can recapitulate the complexity of the tumor ECM, providing more reliable results to mimic TME. Our aim is to fabricate 3D CDMs from human dermal fibroblasts (hDFs) or bone marrow human mesenchymal stem cells (BM-hMSCs) in the presence of macromolecular crowding (MMC) on PLA microparticles (MPs) that can be used as scaffold for reseeding cancer cells to generate tumor models for cancer research.In this study, we cultured hDFs with MPs to produce microtissues. MPs was generated by jet break-up method and functionalized with fibronectin or Collagen-I. hDFs were seeded on the MPs with a spinner flask for 8h. Then, they were leaved in culture for 10-15 days to produce CDM. hDFs were treated with TGF_-1 to differentiate fibroblasts into myofibroblasts and with ascorbic acid to induce collagen synthesis. In addition, the effect of different concentration of Ficoll was assayed. The cellular viability and cell seeding in the MPs were evaluated. The ECM production was evaluated by BCA, hydroxyproline, SEM and immunofluorescence. Values were normalized by the total DNA. Based on our results, Ficoll, TGF_-1 and Ascorbic acid combination increased total protein and total collagen production both in 2D and 3D culture. Total collagen after 10- or 15-days incubation was increased significantly in the presence of Ficoll condition on the fibronectin coated MPs than control group. In conclusion, CDMs are promising biomaterials for establishing 3D *in vitro* tumor models for cancer research, testing anti-cancer drugs and for personalized medicine in the coming years.

POSTER 4 presented by:

NAME: Francesco De Chiara
GROUP: Biosensors for bioengineering
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Micro-IAIver: Artificial intelligence-powered drug screening platform for 3D-bioprinted human liver microspheres

Background and Aims: Non-alcoholic fatty liver (NAFL) is characterized by >5% of steatotic hepatocytes. Its more aggressive form is known as non-alcoholic steatohepatitis (NASH). This latter form is characterized by extended liver inflammation and often accompanied by fibrosis, which may progress to cirrhosis and hepatocellular carcinoma (HCC). The strongest predictor of mortality in NAFL/NASH patients is the advanced fibrosis. Currently, there are no drugs for NAFL/NASH treatment mainly because of the high failure rate of the clinical trial phases. The standard pipeline for the identification of drug candidates lacks accuracy and reproducibility, especially in the preclinical phase. Many *in vitro* models have been developed to recapitulate the pathophysiological conditions of NAFL/NASH *in vitro*. However, these models are difficult (i) to replicate, (ii) to scale to an industrial setting, and (iii) to identify a clear outcome. Here, we use UniINK, a patented 3D printer ink employed for cell encapsulation. We used this technology to recreate a mini-liver using hepatocytes, hepatic stellate cells and Kupffer cells in a high-throughput manner, minimizing the input from the operator. Upon fat treatment, UniINK presents the main signatures of NAFL/NASH in a time-dependent manner, including the fibrosis. These features are identified, analysed, and interpreted by a machine learning approach to calculate the therapeutic index of drug candidates (microl-AI-ver). Method: UniINK is collagen-based ink further crosslinked by tannic acid. HepaRG (human hepatocytes), LX-2 (human stellate cells), and THP-1 (human monocytes) are encapsulated in spheroids using 3D bioprinter and challenged with a mixture of oleic and palmitic acids. At the end of the experiment, the spheroids are passed through a flow-based tomographic microscope and interrogated one-by-one for features of hepatocyte's death, and HSC/THP-1 activation. Results: We developed a robust 3D bioprinting method that fabricates 48k micro-livers/hour with an average cell density of 3M cell/spheroid at cost of €0.80/spheroids. The spheroids are incubated up to one month showing no necrotic area and high viability for untreated cells whereas fat and fibrosis accumulation and cell death in the fat treated condition. The features are classified using k-nearest neighbour (k-NN), support vector machine (SVM), and decision tree to build and implement the dataset. Deep learning networks, such as convolutional neural networks (CNNs) and recurrent neural networks (RNNs) are employed to automatically extract features, group the data, and recognize patterns to make decisions based on beneficial effects of drug treatment. Conclusion: Microl-AI-ver allows: 1) to reduce the variability associated to the operator; 2) to be scalable at the industry level; 3) to spot early signs of fibrosis.

POSTER 5 presented by:

NAME: Maria Demestre
GROUP: Bioengineering in Reproductive Health
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

3D-imaging of mammalian embryo implantation ex vivo

The mouse embryo is central to the study of mammalian development. However, to visualize how the trophoblast, i.e. the embryonic layer that will give rise to the placenta, invades the endometrium during implantation in a 3D context has not been possible yet due to technical constraints. A major setback is that the decidua, a differentiated endometrium surrounding the implanting embryo, is very difficult to surpass by conventional immunohistochemistry techniques. Here, we describe a method whereby using an immunohistochemistry protocol developed for whole mount cleared tissues, and advanced imaging techniques we can obtain 3D reconstructed images of the whole conceptus, i.e. the decidua, the trophoblast, and the embryo. At embryonic day 6.5 after fertilization, we observed the embryo and the trophoblast surrounding it forming a distinctive elongated shape with cell projections, and detached cells invading the surrounding endometrium. This method could be used to characterize trophoblast invasion of the decidua across embryonic developmental stages and serve as a tool to visualize and study any morphological alterations during implantation in a 3D manner.

POSTER 6 presented by:

NAME: Juanma Fernandez-Costa
GROUP: Biosensors for bioengineering
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

A multi-organ-on-a-chip device to study the metabolic crosstalk between muscle and pancreatic islets

Diabetes mellitus is a chronic disease that represents a major public health problem worldwide. Type 2 diabetes (T2D) is the most common form of this disease, accounting for 90-95% of cases of diabetes and is characterized by hyperinsulinemia and insulin resistance. T2D is a complex metabolic disorder that comprises several organs. The pancreas is the organ with a critical role in T2D since in the pancreatic islets, beta-cells produce, store and release insulin. Skeletal muscle is one of the major tissues targeted by insulin and is responsible for maintaining whole-body glucose homeostasis. Understanding the mechanisms that control the metabolic control between skeletal muscle and pancreatic islets is fundamental to developing new molecular drugs to prevent and control this disease. Organ-on-a-Chip (OOC) devices offer new approaches to studying human diseases. In this work, we engineer a new *in vitro* model to study skeletal muscle and pancreas. To this aim, muscle tissues and pancreatic islets have been fabricated and combined in a multi-OOC approach. Moreover, the multi-OOC device was integrated with a Localized Surface Plasmon Resonance (LSPR) sensing module to monitor the insulin and interleukin-6 (IL-6) secretion online and label-free. Using this *in vitro* platform, we have monitored insulin secretion dynamics by the pancreatic islets in response to the skeletal muscle contraction induced by electric pulse stimulation. These results point that this multi-OOC is an important enabling step for diabetes modeling, the study of insulin resistance, and the investigation of drug candidates for therapy, usually performed by long-time and expensive animal experiments. It would open new areas of research on human diabetes disease.

POSTER 7 presented by:

NAME: Xiomara Fernández-Garibay
GROUP: Biosensors for bioengineering
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Xeno-free bioengineered human skeletal muscle tissues

Bioengineered human skeletal muscle tissues have emerged in the last years as new *in vitro* systems for disease modelling. These bioartificial muscles are usually fabricated by encapsulating human myogenic precursor cells in a hydrogel scaffold that resembles the extracellular matrix (ECM). Nowadays, most of these hydrogels are derived from xenogenic sources, such as gelatin from porcine skin, collagen from rat tail, fibrinogen from bovine plasma, or decellularized ECM from porcine muscle. This limits any clinical application of engineered tissues in regenerative medicine due to the possible presence of xenogenic contaminants from animal-derived ECM. Moreover, animal-derived serum in culture media could reduce sensitivity to drug toxicity within *in vitro* testing platforms. These limitations can be overcome by using xeno-free biomaterials and culture conditions, which offer increased relevance for developing human disease models. In this work, for the first time, human platelet lysate-based nanocomposite hydrogels (HUgel) were used as scaffolds for human skeletal muscle tissue engineering in a xeno-free culture. These hydrogels consist of human platelet lysate reinforced with cellulose nanocrystals that allow tunable mechanical, structural, and biochemical properties for the 3D culture of stem cells. Here, we fabricated hydrogel casting platforms for the encapsulation of human muscle satellite stem cells in HUgel around a pair of flexible posts, which act as tendon-like anchoring sites that aid cell alignment and myotube formation. The content of cellulose nanocrystals was modulated to obtain long-lasting scaffolds with high clot retraction properties that promote skeletal muscle tissue formation. We demonstrated that this optimization enhanced matrix remodelling, uniaxial tension, and self-organization of the cells, resulting in the formation of highly aligned, long myotubes expressing sarcomeric proteins. The xeno-free bioengineered human muscles were subjected to a frequency sweep electrical pulse stimulation regime. The electrically stimulated tissues presented twitch or tetanic contractions depending on the applied frequencies. Furthermore, the exerted contractile forces were measured in a non-invasive manner. Overall, our results demonstrated that bioengineered human skeletal muscles could be built in xeno-free cell culture platforms to assess tissue functionality. These tissues could be used as patient-specific models for muscular diseases by incorporating patient-derived platelet lysate and satellite cells. Remarkably, the xeno-free characteristics of this *in vitro* 3D model could also enable the transition into clinical applications for regenerative medicine.

POSTER 8 presented by:

NAME: Ainhoa Ferret Miñana
GROUP: Biosensors for bioengineering
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

A human 3D micro-liver to study the non-alcoholic steatohepatitis-related fibrosis

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disorder worldwide, affecting up to 25% of the adult population. NAFLD ranges from simple steatosis, characterized by fat accumulation within the 5% or more of the hepatocytes, to its most aggressive form, known as non-alcoholic steatohepatitis (NASH). NASH is characterized by liver inflammation, hepatocellular ballooning, and fibrosis. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD. Despite the extent of the problematic, there are still no approved therapies to treat fibrosis to date. Thus, using an *in vitro* system to study the fibrosis mechanisms and the drug response might speed up the drug discovery process. Here, we developed human micro-livers combining hepatocytes (HepaRG), hepatic stellate cells (HSCs) (LX-2), and monocytes (THP-1), the three main cell types of the liver. We used a well-established mixture of gelatine methacryloyl and carboxymethylcellulose as a functional biomaterial for long-term cultures. The 3D micro-livers were challenged with non-esterified fatty acids followed by transforming growth factor beta (TGF-beta) to recreate the pathophysiological phenotype of NASH with medium and high stages of fibrosis *in vitro*. Upon treatment, the micro-livers displayed fat accumulation within the hepatocytes and loss of their metabolic activity. We observed an exacerbated accumulation of fibrotic tissue (collagen) due to the activation of HSCs into fibrogenic myofibroblasts. Moreover, we saw the activation of the immune cells due to the detection of danger signals from injured hepatocytes and activated HSCs. For the first time, we developed a platform to study the fibrosis triggering, release, and accumulation in a standardized *in vitro* platform. Using this tool, we are able to: (i) study the molecular mechanisms involved in the progression of fibrosis in liver disease; (ii) identify new compounds to treat NASH-related fibrosis; (iii) mimic the native microenvironment replacing cell lines with primary or differentiated stem cells; (iiii) upgrade to a high-throughput drug screening platform; (iiiii) scalable at industry level.

POSTER 9 presented by:

NAME: Joseph Forth
GROUP:
INSTITUTION: University College London

Printing Vascularised Scaffolds of Arbitrary Composition using the Granular Jamming Transition

Fabricating vascularised tissue scaffolds with flexible chemical and mechanical properties is essential for producing next-generation tissue models with applications in toxicology, drug design, and disease modelling. Existing approaches either produce scaffolds with simplistic vasculature, exploit angiogenesis to produce vascular networks with poor reproducibility, or offer limited flexibility in extracellular matrix (ECM) formulation. Here, we use 3D printing to produce a biocompatible tissue scaffold incorporating a complex vascular network. We construct a bioprinter for under £500 with resolution comparable to commercially available options. We use this bioprinter to directly write branched vascular patterns into a biocompatible scaffold comprised of gelatine- and hyaluronic acid-derived hydrogels. We further demonstrate the incorporation of norbornene-based chemistries, allowing us to systematically dope the hydrogel matrix with bioactive peptide motifs. Our approach allows for highly flexible formulation of the synthetic ECM and the recapitulation of the rheological properties of the cranial ECM. This further enables us to investigate the fundamental trade-off between scaffold mechanical properties and the resolution of the printed vasculature. Finally, we will discuss preliminary work on incorporating mouse brain astrocytes and endothelial cells into our model.

POSTER 10 presented by:

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

Co-axial 3D bioprinting for Biomimetic Multifiber Skeletal Muscle-based Bioactuators

Recent advances in three-dimensional (3D) bioprinting and tissue engineering have openednew possibilities in the fabrication of bioengineered muscle models able to mimic the complex hierarchical organization and functional properties from the native tissues [1]. The combination of skeletal muscle tissue and artificial elements has led to a wide variety of innovative solutions to create bio-hybrid robotic systems and bioactuators [2] that offer the opportunity to study processes of interest in the biomedical field, such as muscle development and regenerationHowever, one key problem in tissue engineering is the poor oxygen and nutrients supply in the inner regions of the printed scaffold, leading to a reduced cell viability.In our work, we explored co-axial 3D bioprinting [3] as a novel strategy towards overcoming the nutrient diffusion problem by creating individual, non-fused fibers with defined thickness. Therefore, we aim to develop a 3D bioengineered skeletal muscle bioactuator with biomimetic design in terms of structure and functionality. In comparison with conventional 3D-bioprinting,where a single syringe containing the cell-laden bioink is used, in co-axial 3D-bioprinting an outer layer of sacrificial material (pluronic acid in this study) allows a physical confinement on the inner layer (i.e bioink), obtaining thin independent printed fibers that can be hierarchically organized. Such technique is generally implemented in the fabrication of vascular systems [4]. The use of bioprinting techniques allow the fabrication of bioengineered muscle-based actuators that present highly aligned myotubes with contractile capabilities. However, the formation of thinner and individual fibers obtained by co-axial 3D-printing resulted in an enhanced diffusion of nutrients during the muscle maturation process, improving cell differentiation and obtainingstronger bioactuators which present an increased force output in comparison with the actuators fabricated by using conventional printing. After exploring the potential of 3D bioprinting for fabricating 3D bioengineered skeletal muscle bioactuators, our interests are currently focused on exploiting the regenerative capabilities of muscle tissue to integrate self-healing properties to living actuators [5] and create more biomimetic *in vitro* muscle models for biomedical applications.

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POSTER 11 presented by:

NAME: María García Díaz
GROUP: Biomimetic systems for cell engineering
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Stromal-epithelial crosstalk in an immunocompetent model of the intestinal mucosa

Stromal-epithelial crosstalk in an immunocompetent model of the intestinal mucosaMaría García-Díaz, Anna Vila, Carla Arca, Núria Torras, Elena MartínezBiomimetic systems for cell engineering group, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, SpainThe integration of the intestinal epithelium and the stromal compartments is critical for gut homeostasis. In the intestinal stromal compartment, multiple cell types such as mesenchymal cells like myofibroblasts and immune cells like macrophages coexist in a functional equilibrium. The crosstalk between the stromal cells and the epithelium is key to maintain this equilibrium and properly respond to an inflammatory event [1]. Thus, we have developed a hydrogel-based model of the intestinal mucosa encompassing the epithelial, stromal, and immune compartments to reproduce this cellular crosstalk. THP-1 monocytes and/or CCD-18Co myofibroblasts were encapsulated into a hydrogel co-network of polyethylene glycol diacrylate (PEGDA) and gelatin methacryloyl (GelMA). Caco-2 cells were seeded on top to obtain a differentiated epithelial barrier. Inside the hydrogel, the CCD-18Co elongated and interacted with the epithelial cells, promoting the development of the monolayer [2]. The encapsulated THP-1 cells spontaneoulsy responded to the biomaterial by partially differentiating into a macrophage phenotype. This differentiation was significantly enhanced in the presence of the myofibroblasts and/or the epithelial cells, demonstrating a paracrine crosstalk between the different cell types. This immunocompetent model would allow the study of the role of this crosstalk in the modulation of inflammation in diseases such as the inflammatory bowel disease or an intestinal infection.

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POSTER 12 presented by:

NAME: Elena Garreta
GROUP: Pluripotency for organ regeneration
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Exploring the use of human pluripotent stem cells derived kidney organoids during ex vivo normothermic perfusion in porcine kidneys

Normothermic machine perfusion (NMP) of donor organs offers a plausible scenario for improved graft preservation and objective pre-transplant ex-vivo organ assessment. Currently, several works have been directed towards the identification of optimal perfusion formulations for renal NMP. In this collaborative work we seek to explore on the impact of kidney organoid infusion during NMP to evaluate the influence of human pluripotent stem cells (hPSCs) derived kidney organoids as a proof of concept for kidney regeneration ex-vivo. To this end, we developed a new procedure for the derivation of hPSCs kidney organoids in free-floating conditions to scale up organoid production (30.000 organoids per experiment) in a robust manner. Further analyses were conducted including confocal microscopy analysis of major proteins from tubular, glomerular and endothelial kidney compartments together with single cell RNA sequencing to properly dissect kidney organoid cellular identity and composition. Porcine kidneys underwent NMP at 37ºC for 4 hours, using a previously reported perfusion solution in the presence or absence of hPSCs-derived kidney organoids (30.000 organoids per kidney). NMP flow patterns showed stable flow rates among experiments (n=2). Samples were analyzed by conventional immunohistochemistry, flow cytometry analysis, and for the detection of human Alu. Further analysis included the detection of human cells by confocal microscopy analysis using and endogenous reporter for the renal marker WT1 together with the use of a dye as a proxy of human cells detection. Further research is required to continue characterizing these first experiments so that hPSCs-kidney organoids can be exploited as a tool kit for ex vivo cell-based therapy to improve graft function before transplantation.

POSTER 13 presented by:

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

Nociception-Dependent CCL21 Induces Dorsal Root Ganglia Axonal Growth via CCR7-ERK Activation

While chemokines were originally described for their ability to induce cell migration, many studies show how these proteins also take part in many other cell functions, acting as adaptable messengers in the communication between a diversity of cell types. In the nervous system, chemokines participate both in physiological and pathological processes, and while their expression is often described on glial and immune cells, growing evidence describes the expression of chemokines and their receptors in neurons, highlighting their potential in auto- and paracrine signalling. In this study we analysed the role of nociception in the neuronal chemokinome, and in turn their role in axonal growth. We found that stimulating TRPV1+ nociceptors induces a transient increase in CCL21. Interestingly we also found that CCL21 enhances neurite growth of large diameter proprioceptors *in vitro*. Consistent with this, we show that proprioceptors express the CCL21 receptor CCR7, and a CCR7 neutralizing antibody dose-dependently attenuates CCL21-induced neurite outgrowth. Mechanistically, we found that CCL21 binds locally to its receptor CCR7 at the growth cone, activating the downstream MEK-ERK pathway, that in turn activates N-WASP, triggering actin filament ramification in the growth cone, resulting in increased axonal growth.

POSTER 14 presented by:

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

Cardiac extracellular matrix hydrogels for injectable scaffold fabrication

In first-in-man studies, the use of myocardial extracellular matrix (ECM) derived materials for the treatment of myocardial infarction has already been shown to be safe, feasible and even allow suggestions of improvements to patient outcomes.1 Increased neovascularization, reduced fibrosis and increased cardiac muscle were seen in animal models, along with greater cardiac differentiation of stem cells *in vitro*.2-4 Utilising a well-defined decellularisation method, involving cryoslicing and treatment with several solvents, we isolated cardiac ECM from porcine tissue. Sufficient removal of cells and DNA that may cause an immune response must be achieved by tuning the concentration of the solvents and the duration of each treatment step, while minimally affecting the proteoglycan contents and the gelation of the ECM. Assessment of the physical properties by rheology and the effect on the growth of cells will also be carried out.Through this project, ECM powder with less than 150 ng DNA per mg of ECM was achieved without significantly reducing the glycosaminoglycan content. This ECM also formed hydrogels after digestion with pepsin and incubation at 37ºC that were well tolerated by the cells tested (rat cardiomyoblasts (H9c2) and human umbilical vein endothelial cells (HUVEC)). The physical properties of the gel matched those achieved in the literature.

ACKNOWLEDGEMENTS:
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POSTER 15 presented by:

NAME: Nina Kostina
GROUP: Bioinspired Interactive Materials and Protocellular Systems Group
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Ionic combisomes: A new class of biomimetic vesicles to fuse with life

The fabrication of biomembranes that closely replicate the properties and dynamic functions of natural cell membranes is a major challenge in the development of synthetic cells and their applications. Herein, we introduce a new concept for fully synthetic cell membranes based on a new family of amphiphilic comb polymers which self-assemble into vesicles, termed ionic combisomes (i-combisomes). These combs consist of a polyzwitterionic backbone to which lipid-like hydrophobic tails were linked by electrostatic interactions. Using a combination of molecular simulations, optical, electron, and force microscopies, we screened the self-assembly of a library of combs with tailored structural variations to unravel the structure–property relationship. We discovered that a high density of hydrophobic tails drives the formation of the membrane's core and forces the hydrophilic backbone into a rod conformation with nematic-like ordering confined to the water-bilayer interface. This specific molecular organization led to membranes that combine the biomimetic thickness, lateral mobility, and flexibility of liposomes with the stability of classic polymersomes. Such high mimicry of biophysical properties and the ability to locally remodel the molecular topology of their components allows them to harbor functional components of natural membranes and to fuse with living bacteria to "hijack" their periphery. This offers a powerful set of tools for designing the chemical and biological composition of the i-combisome membrane, providing a platform for fundamental studies, equipping synthetic cells with advanced functions and (bio)technological applications that go beyond biological limits.

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POSTER 16 presented by:

NAME: Nina Kostina
GROUP: Bioinspired Interactive Materials and Protocellular Systems Group
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Synthetic dendrimersome membrane could lead to self-replicating artificial cells

The integration of active cell machinery with synthetic building blocks is the bridge towards developing synthetic cells with functions beyond life. Replication and division are among the most important challenges to endow artificial cells with "livingness". In nature, the E. coli divisome is one of the simplest yet extremely elegant ways to divide cells consisting of two coordinate elements: a constriction and a positioning system. In the former, FtsZ proteins assemble into the Z-ring exerting a force to sever the membrane. The Z-ring must be positioned at the equator, a task that is controlled by the Min system. Currently, the Min system has only been reconstituted in liposomes presumably due to the large differences in physical properties of synthetic cell membranes.[1] This seriously limits the applications in which these synthetic cells can be used in terms of stability and functionalizability. Here we show that new biomimetic dendrimersomes assembled from zwitterionic Janus dendrimers supported the reconstitution of an active bacterial divisome.[2] This was possible by programming the strength and dynamics of membrane-divisome interactions and reproduce its dynamic behaviour. This constitutes an important breakthrough in the assembly of synthetic cells with biological elements, as tuning of membrane-divisome interactions is the key to engineer emergent biological behaviour from the bottom-up

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POSTER 17 presented by:

NAME: Maria José López Martínez
GROUP: Bioinspired Interactive Materials and Protocellular Systems Group
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

A phosphocholine-based Janus dendrimer for the next level of biomimicry in dendrimersomes

Bottom-up synthetic biology aims at building a synthetic cell by the assembly of a minimal set of rationally designed components.[1] Without the complexity of natural cells these synthetic cells offer a means to unravel fundamental questions in biology and to improve current biotechnologies. One of the most important cell components is its membrane. To prepare synthetic membranes, scientists commonly turn to nature's choice of membrane-forming phospholipids and assemble them into vesicles. However, without their constant repair and replenishment as in cells their lack of chemical and physical stability is a limiting factor in many applications. Amphiphilic Janus dendrimers (JDs) that self-assemble into dendrimersome vesicles (DSs) are a recent addition to membrane-forming amphiphiles, which combine the biomimetic physical properties of lipids with an increased stability. However, most DSs rely on a polyol-based hydrophilic periphery and not on biomimetic phosphocholine.[2] This alters the behavior of incorporated membrane proteins or their interactions with the environment. To improve the biomimicry, we designed a zwitterionic JD bearing a phosphocholine group, which faithfully recapitulates the cell membrane in thickness, flexibility and mobility while being resilient to harsh irradiation/temperature conditions and displaying improved self-healing properties. This allowed us to readily fabricate hybrid DSs that harbor components of natural membranes, including lipids, proteins, glycans and even structure-directing lipids to create raft-like domains. Despite their synthetic nature, the DSs were compatible with biological media and therefore offer an effective platform to create synthetic cells. Ultimately, we utilized these synthetic cells to mimic sensing, fusion and motility of their natural counterparts.

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POSTER 18 presented by:

NAME: Lluís Mangas Florencio
GROUP: Molecular Imaging for Precision Medicine
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Development of a MR-compatible bioreactor platform for hepatocellular carcinoma metabolic analysis in real-time using a 60 MHz benchtop NMR Spectrometer

Hyperpolarized nuclear magnetic resonance (NMR), for example using dissolution dynamic nuclear polarization (DNP), provides over 10.000-fold increase in signal intensity compared to conventional NMR acquisitions. Owing to this great signal enhancement, hyperpolarized NMR has become a non-invasive technique that allows the analysis of cellular metabolism *in vivo* and in real-time by enhancing the spin polarization of 13C nuclei in metabolites of interest and following their metabolic conversion in live subjects and samples. While this technique has already started its transition into clinical settings as a diagnostic tracking tool for cancer stages and metabolic anomalies detection, hyperpolarized NMR is still widely used in a preclinical setting. In-cell studies by NMR are often limited by poor signal intensities (long acquisitions), the bore size and geometry of the NMR magnets (samples analyzed in tubes of 5-, 10- or 20-mm of diameter), and the cell model used (mainly cell suspension). Here we present a strategy to overcome these three limitations. We have designed a 3D printed platform for longitudinal hyperpolarized NMR experiments on a 3D cell model using conventional NMR spectrometers. Our platform allows for cell maintenance by constant and controlled media perfusion, as well as injection of the hyperpolarized substrate into the sample for realtime metabolic studies of 3D constructs. The use of 3D cells, cultured in cell culture vessels and moved into NMR tube before the analysis, improves the biological relevance of the experiments, and reduces cellular stress during manipulation.

POSTER 19 presented by:

NAME: Domingo Marchan del Pino
GROUP: Bacterial infections: antimicrobial therapies
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

New broad-host-range promoter probe vectors for gene expression analysis by expressing multiple fluorescent proteins

Bacterial processes are conditioned by the genic expression from the microorganisms that are under specific environment condition. For that, the analysis of the different parameters which can affect the general phenotype of the bacteria is a good way to study this phenomena. Reporter genes like fluorescent proteins (FP) allowed the study of gene promoter activities and it'ss regulation *in vivo* or in *in vitro* models. Nowadays several FPs are used for different objectives due to the fact that they are a non-invasive tool in molecular biology. Transcriptional fusions between a promoter of interest and this FPs are a good way to measure the gene activity and it can be done in a plasmid. These are known as promoter-probe vectors, but it'ss use requires the presence of a selection pressure for its stability and that is usually conferred by an antibiotic addition. For that it is desirable to integrate the promoter-reporter fusions in the bacterial genome to avoid the requirement of antibiotic addition for the plasmid maintenance and integrity. Homologous recombination using suicide vectors, and the utilization of mobile elements like Tn5 transposon system are two main techniques used to insert foreign DNA fragments into the bacterial genome. In this work, a new generation of promoter-probe and transposon-delivery vectors namely pETS-IBECGLOW were designed and developed. These vectors contain the genes encoding for different FPs, under the regulation of the three promoters of Pseudomonas aeruginosa ribonucleotide reductases (RNRs) classes. The plasmids are designed to improve the promotor-dependent FPs expression and preventing the non-specific background leaking.

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POSTER 20 presented by:

NAME: Andrés Marco Giménez
GROUP: Pluripotency for organ regeneration
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

CRISPR-engineered kidney organoids to study gene function in renal development and disease

Kidney organoids derived from human pluripotent stem cells (hPSCs) have proved to recapitulate, in a high extent, the development, multicellular architecture, and physiology of the human embryonic kidney. To perform genetic studies in these models, methods for efficient and controllable genetic manipulation of hPSCs are needed. To fulfil this need, this work has focused on the generation of a platform allowing for the inducible expression of Cas9 (iCRISPR2 (iC2)) platform). This system allows the high-throughput generation of stable and inducible knockout (KO) hPSC lines, precise knockin (KI) hPSC lines, and reporter hPSC lines. Resulting iC2-engineered hPSCs were differentiated into kidney organoids to study gene function in kidney development and disease. Early phenotypic characterization of kidney organoids derived from stable and inducible LHX1 KO hPSCs showed that LHX1 function is required for early steps of nephron formation (nephrogenesis). Likewise, lack of PAX2 impaired nephrogenesis by mainly affecting the formation of proximal tubular-like structures. On the other hand, organoids derived from hPSCs carrying KO and patient-specific mutations at the WT1 gene showed renal differentiation impairment and the acquisition of relevant kidney disease-related phenotypes. Similarly, tubular-like cells derived from kidney organoids with VHL depletion showed mitochondrial respiration decrease, paralleling one of the major hallmarks of clear cell renal carcinoma. Finally, differentiation of WT1 and NPHS2 hPSC reporter cell lines validated their utility to isolate cell populations expressing these marker genes for their further characterization. On balance, this work demonstrates that CRISPR engineered kidney organoids represent an unvaluable model system to dissect gene function in human kidney development, physiology and disease *in vitro*.

POSTER 21 presented by:

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

Muscle-on-a-chip platform highlights a significant drop in muscle function post-treatment with CFS sera

Chronic Fatigue Syndrome or Myalgic Encephalomyelitis is a long-term, debilitating condition characterized by extreme tiredness. The patients usually have a history of viral infection and experience severe post-exertional malaise. At the moment, contemporary literature offers conflicting evidence pertaining to disease diagnoses, progression and treatment. Our research aims to understand the patho-mechanism of the disease by using 3-D *in vitro* skeletal muscle tissues. To the best of our knowledge no study has yet been conducted to understand the impact of patient serum on skeletal muscle function using *in vitro* 3-D platforms. Consequently, a lot of ground is yet to be explored. The said 3-D tissues were fabricated using Matrigel-Fibrinogen encapsulated satellite cells on a PDMS support. The preliminary comparative functional and structural analyses of tissues treated with patient and healthy sera after Electric Pulse Stimulatory (EPS) training suggests a significantly weaker specific force of contraction for tissues treated with patient sera. However, no significant structural difference in myotubes was observed. The mechanism of disease progression and manifestation, therefore, does not appear to be atrophic but instead could be metabolic in nature. The utility of this platform is an important step towards understanding patient specific sample variability and personalized response to multiple drug testing regimens.

POSTER 22 presented by:

NAME: Adria Noguera Monteagudo
GROUP: Biomaterials for regenerative therapies
INSTITUTION: Universitat de Barcelona

Towards Angiogenesis-On-A-Chip

Angiogenesis is essential for organ growth and repair and is very important as it is associated with a variety of health problems. Advances in microfluidic technology have enabled *in vitro* microenvironment replication to study the mechanism of angiogenesis through *in vitro* models. This work investigates the design and operation of a microfluidic chip to study the angiogenesis involving isolated endothelial cells (EC). In this direction, a characterization of the used extracellular matrix (ECM), fibrin hydrogel, was performed, both physically, by rheology; and structurally, by staining the hydrogel. To study the angiogenesis process, the migration of EC was compared with different concentration gradients of the angiogenic signal like VEGF. Moreover, by characterizing and controlling the physical and structural parameters of the ECM, it is possible to study the influence of these properties on angiogenesis. In terms of cellular results, it was observed that the presented design allows the generation of an angiogenic factor gradient that stimulates cell migration. In some initial experiments, it was observed that increasing the concentration of VEGF increases cell migration. These results have been used to develop a mathematical model that simulates the migration of EC chemotactically migrating through a fibrin hydrogel considering the ECM and chemotactic factor distribution. This model focuses on modeling budding dynamics and morphology, providing a new framework for understanding cell migration and the influence of biomaterial structure.

POSTER 23 presented by:

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

Bioprinted hydrogel-based model of the tumor microenvironment in colorectal cancer

In colorectal cancer (CRC), the second most common cancer in humans, approximately 50% of patients develop metastasis [1]. Metastasis triggers when cells from the primary tumor intravasate into the blood circulation, circulate in the bloodstream, and extravasate into a metastatic region by migrating through the vessel walls [2]. This cascade of events is driven by many factors such as the tumor microenvironment (TME) that, especially in CRC, has a key role on the disease progression and dissemination [3]. Thus, we have developed 3D hydrogel-based model using an innovative set-up based on digital light processing (DLP) bioprinting that mimics the tumoral and vascular microenvironment of CRC to study metastatic events.To mimic the TME, we bioprinted stromal fibroblasts and cancer spheroids inside the hydrogel and grow the endothelial barrier on top of the model. To this end, we used a bioink composition based on polyethylene glycol diacrylate (PEGDA) and gelatin methacryloyl (GelMA). Human intestinal fibroblasts (HIFs) were embedded in the hydrogel discs to mimic the intestinal stromal microenvironment. Embedded cells showed high viability after printing and cells elongated within the hydrogel. However, fibroblasts showed impaired migration within the hydrogel bulk. In order to ease migration and elongation of stromal cells, we combined our bioink with fibrinogen to obtain an interpenetrating polymer network of PEGDA/GelMA-fibrin after printing. In our fibrin bioink, HIFs showed higher cellular viability, higher cellular elongation, and an increased migration rate.To mimic the endothelial barrier, primary endothelial HUVEC cells were grown on top of hydrogel discs. They form a monolayer with proper barrier characteristics up to 7 days after seeding, as demonstrated by TEER measurements and immunostaining of endothelial markers such as VE-cadherin. The presence of HIFs enhanced the endothelial monolayer formation, whereas the presence of fibrin promoted the endothelial sprouting towards the hydrogel bulk. To complete the CRC model, GFP-labeled SW480 tumor cell line was grown into spheroids and encapsulated inside the hydrogels in combination with HIFs. The growth and interaction with the stromal cells were monitored over time, as well as their transepithelial mesenchymal transition with differential expression of E-cadherin and vimentin markers. Taking an innovative and different approach, we developed a 3D hydrogel model using a biofabrication technique based on visible light. This 3D model supported HIFs migration, HUVEC monolayer formation and the interaction between HIFs and spheroids. The proposed hydrogel-based model that recapitulates some of the key elements of the TME in CRC can be used to evaluate the triggering elements of metastasis in CRC.

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POSTER 24 presented by:

NAME: Alessandro Ronzoni
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Regulating gene expression in different tissues: How to deliver drugs to the brain using translational regulation to reduce off-targeting

Delivering mRNA to brain endothelial cells is a promising therapeutic approach to tackle brain disorders. However, unspecific uptake of mRNA by peripheral endothelial cells, particularly liver endothelial cells, limits the specificity of mRNA expression.We are developing a strategy to enhance mRNA expression in BECs while inhibiting it in liver endothelial cells. To do so, we exploit the difference between the physiology of brain endothelial cells, characterized by a low proliferation rate, and the liver endothelial cells, which show a faster proliferation.We base our work on a mechanism of regulation of translation driven by microRNA interaction with target mRNA sequences. We are following published data partially showing the activity of microRNAs in the regulation of the translation of target mRNAs in model cell lines. We observed that the synthetic microRNA miRcxcr4 represses translation of target mRNA in proliferative cells, yet it upregulates translation in non-proliferating cells. We are examining this mechanism in endothelial cells from different organs.We aim to exploit this mechanism to achieve differential mRNA expression in brain vs liver endothelial cells. Using mRNA target sequences for miRNAs able to regulate mRNA expression would lead to an increase in the specificity of the system, enhancing the expression on the BBB and inhibiting expression in other cell types (i.e. endothelial cells of the liver), which constitute off-targets that hinder the use of this system.

POSTER 25 presented by:

NAME: Julia Sala Jarque
GROUP: Molecular and cellular neurobiotechnology
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Does PRPC have a major role in the progression of TAU pathology?

Tauopathies are a group of neurodegenerative diseases (e.g., Alzheimer'ss disease (AD)) characterized by the accumulation and deposition of the Tau protein into amyloid structures. The progression of Tau pathology in AD follows a predictable, hierarchical, and stereotyped pattern between anatomically connected areas of the brain. Thus, it is thought that pathological Tau (pTau) protein engages in a self-amplification process known as "seeding" followed by a trans-cellular "spreading" of the disease. However, the precise molecular mechanisms involved remain elusive.Recently, the cellular prion protein (PrPC) has been proposed to mediate A_-amyloid progression in AD and a-synuclein in Parkinson's disease. Yet, less is known about its role in the induction and progression of Tau pathology. In this regard, we inoculated the parietal cortex and corpus callosum of wild-type, Tau knock-out (KO), PrPC KO (Zürich III), and PrPC anchorless (Tg44) mice with samples from AD patients. All mice models, except for the Tau KO, revealed similar amounts of pTau aggregates and spreading at 3 and 6 months post-inoculation. Using immunohistochemical techniques, we have determined that such aggregates mainly contain murine endogenous Tau being positive for histopathological pTau markers such as AT8 and PHF-1. Thereby, our preliminary findings suggest that PrPC does not seem to have a crucial role in the initialization and/or progression of Tau pathology in our tested models, and other mechanisms might run in parallel. Current experiments are directed to determine these alternative processes and whether they are linked to PrPC

POSTER 26 presented by:

NAME: Eduardo Sesma
GROUP: Bioengineering in Reproductive Health
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Use of PLASTEM® as a cell culture supplement for cell therapy manufacturing

Introduction & Aim: Cell therapy offers potential for the treatment of a range of diseases and a regenerative medicine solution to many clinical needs related to ageing. Most used supplements in cell therapy manufacturing, fetal bovine serum (FBS) and human platelet lysate (HPL), both present challenges for supply and regulatory compliance. PLASTEM® is a novel plasma-derived supplement, with a high safety profile, consistency, and scalable production, and could represent an alternative as a cell culture supplement.Methods: PLASTEM® performance as a culture supplement for cell therapy manufacture was assessed. Human Mesenchymal Stromal Cells (hMSC) were expanded with PLASTEM® (plus a 10x reduced amount of HPL), FBS or HPL. Cell growth rate, immunophenotype, multipotentiality and immunomodulation properties were compared.Results: hMSCs cultured with PLASTEM®+0.5% HPL, expanded at a comparable rate to cells grown in FBS supplemented media. They also retained multilineage differentiation capacity and the expression of specific immune markers. In immunomodulation assays, human peripheral blood mononuclear cells (PBMC) co-cultured with hMSCs (previously expanded with PLASTEM® + 0.5% HPL), exhibited a significant percentage inhibition of PBMC proliferation. Conclusion: PLASTEM® is a non-xenogenic supplement that, combined with growth factors, acts as a competitive and robust supplement for cell culture.

POSTER 27 presented by:

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

DMD-on-a-chip: a platform to accelerate drug testing for Duchenne Muscular Dystrophy using a functional patient-derived 3D skeletal muscle model

Duchenne muscular dystrophy (DMD) is the most prevalent neuromuscular disease diagnosed in childhood. It is characterized by a progressive degeneration of skeletal and cardiac muscles, caused by the lack of dystrophin protein. The absence of this structural protein leads to the fragility of the sarcolemma, and muscle fibers are damaged during their contraction. To date, there is no cure available for patients, even though there are several molecules in drug development. However, due to the limitations of preclinical research, the success rate of drugs remains low. In this work, intending to accelerate drug discovery for DMD, we developed an innovative organ-on-a-chip (OOC) platform to faster evaluate anti-DMD treatment candidates. This OOC consists of a microfluidic device that can sustain the culture and electrical pulse stimulation (EPS) of up to six patient-derived 3D functional skeletal muscle tissues. Moreover, it allows the monitorization of drug administration and its integration to sensing platforms to obtain data in a real-time and non-destructive manner. The properties of the microfluidic platform were explored using theoretical simulations, and the device was fabricated and validated. On the other hand, the 3D *in vitro* model of DMD is developed by the encapsulation of myogenic precursors in a fibrin-composite matrix using PDMS casting mold. It incorporates two flexible T-shaped pillars that provide continuous tension to the tissue, thus allowing the orientation of the muscle fibers. These posts served as anchoring points and allowed an easy evaluation of the contractibility of the muscles. After seven days of differentiation, DMD tissues expressed mature myogenic markers and showed functional phenotypes as they responded to electrical pulse stimulation (EPS) by contracting. Using this strategy, we identified DMD-related functional phenotypes that are similarly present in patients, such as loss of myotube integrity due to the sarcolemma instability and decrease of muscle force. This is of high relevance for a precise evaluation of therapeutic responses using the DMD-on-a-chip device. Finally, the applicability of this dystrophic skeletal muscle model in evaluating therapeutic compounds was explored using anti-DMD drug candidates, such as Ezutromid. Taking all these considerations, this work shows that OOCs have great potential to be especially valuable in the discovery and development of drugs to treat DMD and other neuromuscular disorders.

POSTER 28 presented by:

NAME: Karen Isabel Wells Cembrano
GROUP: Molecular and cellular neurobiotechnology
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Biomimetic *in vitro* platform for the study of functional and biochemical effects of myasthenia gravis patient serums

Myasthenia gravis (MG) is an autoimmune disease of the neuromuscular junction (NMJ), which causes muscle weakness of varying severity. Around 80% of patients present autoantibodies directed against the acetylcholine receptor (AChR), although several other targets have been described, including muscle-specific kinase (MuSK, with autoantibodies present in 5% of patients), and other proteins present in the endplate or contractile machinery. There are relevant clinical differences between MG patients of different serologies and even different antibody titers. Thus, the mechanisms of action of each antibody subtype and their relation to symptoms has not been completely elucidated.Using a biomimetic *in vitro* model, we aimed to recreate the effect of MG patient serum autoantibodies on the NMJ. We used immortalized human myoblasts cultured in a biomimetic hydrogel placed between two anchor points, which allows the cells to form an aligned and compacted structure similar to human muscle. After differentiation, the resulting muscle bundle shows formation of endplates, which makes them responsive to acetylcholine stimulation. Myosin heavy chain (MHC) and sarcomeric alpha-actinin (SAA) staining confirm contractile maturity of the bundles. To study functional alterations induced by serum treatment, we analyzed calcium transients in GCaMP6+ muscle after application of anti-AChR, anti-MuSK, or control serum. In parallel, we analyzed the biochemical effect of the serums on AChR and MuSK, by immunostaining and western blot techniques. Our preliminary results show an almost complete loss of AChRs at the endplate after treatment with anti-AChR serum, as well as functional differences in calcium transients.

POSTER 29 presented by:

NAME: Lena Witzdam
GROUP: Bioinspired Interactive Materials and Protocellular Systems
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Kill&Repel Coatings: the Marriage of Antifouling and Bactericidal Properties to Mitigate and Treat Wound Infections

Wound infections originate when exogenous or endogenous bacterial pathogens can circumvent the barrier of the wound dressing and invade the wound bed. Bacterial colonization causes inflammation, stalls the healing process, and carries the risk of dissemination to other tissues. In addition, current antimicrobial dressings fail to resolve an infection once it has been established because debris of the killed bacteria rapidly accumulates on their surface and hampers the antimicrobial action. Faced with this challenge, we developed hybrid synthetic-natural water-soluble macromolecules that self-assemble onto the surface of dressings to generate an antifouling brush functionalized with endolysin, a bactericidal enzyme that poses no harm for eukaryotic cells (Kill&Repel coatings).[1] By exhibiting repellent and bactericidal properties, it limits the initial stages of bacterial adhesion onto the dressing, thus reducing bacterial load. If ,however, bacteria manage to surpass the repellent barrier, the endolysin is capable of killing them upon contact. Cell debris is subsequently repelled. The coating consists of two molecules, one that imparts antifouling properties and another bearing bactericidal activity. The antifouling molecules consist of N-(2-hydroxy-propyl)methacrylamide polymer grafted from the liquid chromatography peak I peptide (LCI-eGFP-pHPMA). Bactericidal activity was achieved by producing a fusion construct of LCI with a highly active endolysin (LCI-EndLys). The Kill&Repel coating was formed by physisorption of both hybrids from a dilute aqueous solution onto the surface of a wound dressing. We showed that the adsorption of LCI-eGFP-pHPMA led to segregation of pHPMA at the periphery of the surface, resulting in a brush-like coating which effectively prevented the fouling from proteins and bacteria. Remarkably, the activity of immobilized LCI-EndLys was higher than that of the molecularly dissolved one, suggesting that the co-adsorption with pHPMA had a boosting effect on LCI-EndLys. This was furtherevidenced by the coating being able to reduce concentration of planktonic bacteria (OD550=0.8) by over 92% and far more efficient than free LCI-Endlys (50%). Similarly, it decreased sessile bacterial colonization on agar plates by 96%. Moreover, it prevented the unspecific adhesion of skin cells, colonization of the dressing by bacteria, as well as debris and was fully innocuous to human cells. We designed a Kill&Repel coating for wound dressings, which synergistically combines the ability to repel pathogens with a bioorthogonal bactericidal strategy that causes no harm to eukaryotic cells. This strategy allows for the targeted killing of bacteria and a self-cleaning mechanism rendering the modified surface an inexhaustible antimicrobial strategy. Thus, this strategy opens a revolutionary approach for protecting and treating an infected wound in a safer and more efficient manner.

POSTER 30 presented by:

NAME: Maria Victoria Batto
GROUP: Associated Researcher
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Relationship between collagen and EMT/metastasis in lung cancer

Lung cancer (LC) is the first cause of cancer related death worldwide. A common hallmark in LC is a desmoplastic/fibrotic stroma rich in fibrillar collagens. Intriguingly, we recently reported that the amount of fibrillar collagens is associated with bad prognosis independently of the current gold standard based on TNM staging. Likewise, fibrillar collagen genes have been found in metastatic gene signatures. Since metastasis has been associated with the ability of some cancer cells to undergo intermediate EMT, our goal was to study the relationship between fibrillar collagens and EMT/metastasis in LC. We first conducted survival analysis of a panel of collagen-related genes in LC using the TCGA database, confirming their expression in RNA-seq data from tumor-associated fibroblasts (TAFs) derived from LC patients, and subsequently performed correlation analysis between these collagen genes and markers of EMT or metastasis. Among 24 collagen-associated genes, 15 were consistently associated with bad prognosis, and 12 of them were strongly expressed in lung TAFs from our cohort. We found a strong correlation between COL1A1, COL3A1, ADAMTS2 and 2 markers of intermediate EMT. Likewise, we found a strong correlation between the same 3 collagen-genes and 3 metastasis markers. Our results shed light on the tumor-promoting effects of fibrillar collagens in LC indicating a strong correlation between collagen with intermediate EMT and metastatic genes, supporting a causal relationship.

POSTER 31 presented by:

NAME: Yolanda Castillo Escario
GROUP: Biomedical signal processing and interpretation
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Convolutional Neural Networks for Apnea Detection from Smartphone Audio Signals

Although sleep apnea is one of the most prevalent sleep disorders, most patients remain undiagnosed and untreated. The gold standard for sleep apnea diagnosis, polysomnography, has important limitations such as its high cost and complexity. This leads to a growing need for novel cost-effective systems. Mobile health tools and deep learning algorithms are nowadays being proposed as innovative solutions for automatic apnea detection. In this work, a convolutional neural network (CNN) is trained for the identification of apnea events from the spectrograms of audio signals recorded with a smartphone. A systematic comparison of the effect of different window sizes on the model performance is provided. According to the results, the best models are obtained with 60 s windows (sensitivity=0.72, specificity=0.89, AUROC=0.88). For smaller windows, the model performance can be negatively impacted, because the windows become shorter than most apnea events, by which sound reductions can no longer be appreciated. On the other hand, longer windows tend to include multiple or mixed events, that will confound the model. This careful trade-off demonstrates the importance of selecting a proper window size to obtain models with adequate predictive power. This paper shows that CNNs applied to smartphone audio signals can facilitate sleep apnea detection in a realistic setting and is a first step towards a machine learning method to assist sleep technicians for automatic sleep apnea screening.

POSTER 32 presented by:

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

NAFLD-sarcopenia crosstalk on a chip: *in vitro* model with nanoplasmonic biosensing

Sarcopenia is a skeletal muscle (SM) insufficiency and failure, which seriously affects mobility, nutritional status, and overall independence of the patients. It is characterized by low muscle strength and quality of the SM. Being a relatively common disease, it comprises a wide range of short and long-term adverse effects. Although it has been primarily related to age, sarcopenia may be associated with other evident factors like metabolic disorders, liver, and kidney damage [1]. Non-alcoholic fatty liver disease (NAFLD) is the most common hepatic disorder worldwide and it has been shown to affect other organs and tissues. Interestingly, NAFLD and sarcopenia show not only an important association at a clinical level but also share linking metabolic pathways, which is why they become interesting to relate in-vitro. For this purpose, organs on chip (OOC) represent an ideal strategy to model the interaction between both diseases. OOC contains miniature tissues inside a microfluidic chip that can maintain a microenvironment similar to the native. This work aims to provide an OOC device hosting 3D models of both human hepatocytes and SM. After reproducing the NAFLD phenotype in the liver model, the culture medium will be directed into the SM chamber to evaluate its effect on differentiated myotubes. Target biomarkers will be measured by antibody-based nanoplasmonic biosensing.

[1] Lancet 2019; 393:2636-46

POSTER 33 presented by:

NAME: Celia Mallafre Muro
GROUP: Signal and information processing for sensing systems
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Study of quality controls for stability check of the ROIs of a ketones mixture in different GC-IMS measurement campaigns

GC-IMS is a very good complementary technique to traditional GC-MS, that presents some advantages, but also, some disadvantages such as misalignments produced by many parameters affecting the equipment stability. The reproducibility of the measures has been studied in two different measurement campaigns with a set of automatized quality control parameters. Figures of merit from one region of interest present in the samples show that the saturation and asymmetry do not change between measurement campaigns, but the volume and area of the total ion spectra change. A correction of these changes between batches should be developed.

POSTER 34 presented by:

NAME: Albert Parra
GROUP: Bioengineering in Reproductive Health
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Artificial Intelligence applied to hyperspectral imaging clasifies embryos and oocytes based on their metabolic profile

To improve the results in in-vitro fertilization (IVF), and avoid physical, emotional and financial burden to the families that cannot achieve pregnancy, the development of a reliable, non-invasive way to assess oocyte and embryo quality becomes increasingly important. We developed a new Hyper-Spectral (HS) imaging method to measure intrinsic metabolic signals of oocytes and embryos in a non-invasive way. We combine multiphoton and HS imaging to provide a safe illumination source to the sample and cover a wide range of the spectrum ~400-to-700nm allowing the simultaneous measurement of 6+ relevant metabolites from the embryo/oocyte biology. Then, a novel Artificial Intelligence (AI) pipeline was designed to classify the embryos and oocytes, subjected to different conditions, from the 4D HS images. RESULTS: A set of mouse embryos cultured in different conditions were HS imaged and several embryologists evaluated the corresponding brightfield images of the test set of embryos. Using brightfield images, human graders correctly classified a low percentage of the good and bad samples compared to the AI algorithm. A similar approach was conducted on mouse oocytes obtained from young or old females. The metabolic information is a robust biomarker for embryo and oocyte classification and combined with the developed HS-AI method has the potential to offer a safe, fast, reliable, non-invasive and direct measurement of the oocyte/embryo physiology.

POSTER 35 presented by:

NAME: Daniel Romero
GROUP: Biomedical signal processing and interpretation
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Predicting 6-minute walking test outcomes in patients with chronic obstructive pulmonary disease without physical performance measures

Chronic obstructive pulmonary disease (COPD) requires a multifactorial assessment, evaluating the airflow limitation and symptoms of the patients. The 6-min walk test (6MWT) is commonly used to evaluate the functional exercise capacity in these patients. This study aims to propose a novel predictive model of the major 6MWT outcomes for COPD assessment, without physical performance measurements. Cardiopulmonary and clinical parameters were obtained from fifty COPD patients. These parameters were used as inputs of a Bayesian network (BN), which integrated three multivariate models including the 6-min walking distance (6MWD), the maximum HR (HRmax) after the walking, and the HR decay 3 minutes after (HRR3). The use of BN allows the assessment of the patients' status by predicting the 6MWT outcomes, but also inferring disease severity parameters based on actual patient's 6MWT outcomes. Results: Firstly, the correlation obtained between the estimated and actual 6MWT measures was strong ($R = 0.84$, MAPE = 8.10% for HRmax) and moderate ($R = 0.58$, MAPE = 15.43% for 6MWD and $R = 0.58$, MAPE = 32.49% for HRR3), improving the classical methods to estimate 6MWD. Secondly, the classification of disease severity showed an accuracy of 78.3% using three severity groups, which increased up to 84.4% for two defined severity groups. We propose a powerful two-way assessment tool for COPD patients, capable of predicting 6MWT outcomes without the need for an actual walking exercise. This model-based tool opens the way to implement a continuous monitoring system for COPD patients at home and to provide more personalized care.

POSTER 36 presented by:

NAME: Ona Baguer Colomer
GROUP: Cellular and molecular mechanobiology
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Role of Nuclear Mechanics in Regulating the Mechanosensitivity of Pancreatic Cancer Cells

Pancreatic ductal adenocarcinoma (PDAC) is characterised by high genomic instability and poor clinical outcome. The extensive stromal remodelling and stiffening found in this disease, even at the preneoplastic lesions, appear to be key in its progression; however, little is known about the role of mechanical stimuli in the rise of such aggressive tumours. To better understand the effects of physical forces on PDAC cells, we want to focus on the cell nucleus. This organelle that protects and organises the genome also acts as a mechanosensor: mechanical forces from the microenvironment are transmitted to the nucleus, where they can deform it and impact on cellular activity by affecting nuclear transport of transcription factors, cell migration, or DNA status. This capacity to sense and respond to forces depends on nuclear stiffness and tension, two mechanical properties dysregulated in PDAC cells that lead to aberrant nuclear shapes. We hypothesise that nuclear mechanosensitivity occurs and is relevant in PDAC, which raises the following questions: i) how do nuclear mechanics regulate the mechanosensitivity of pancreatic cancer cells? ii) how is this associated with the malignancy of cells? To answer these questions, we will tune the chromatin condensation state, the nuclear lamina levels, and the cytoskeleton organisation, the three major contributors to the nuclear mechanics, and submit these pancreatic cancer cells to different mechanical stimuli to assess how they behave. By doing that, we expect to bring insight into the impact of physical forces in the progression of PDAC.

POSTER 37 presented by:

NAME: Alejandro Bernardo Suárez
GROUP: Associated Researcher
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

SMAD3 in tumor associated fibroblasts drives enhanced fibroblast accumulation in lung adenocarcinoma through increased migration

A hallmark of lung cancer and other solid tumors is a stiff fibrotic microenvironment rich in activated tumor-associated fibroblasts (TAFs). TGF-_1 is an efficient fibroblast activator frequently upregulated in lung cancer. Intriguingly, we previously reported a larger accumulation of TAFs in lung adenocarcinoma (ADC) compared to lung squamous cell carcinoma (SCC), although the underlying mechanobiology remain elusive. TAF accumulation is largely contributed by the proliferation and/or migration of resident fibroblasts. Notably, SMAD3 is an important transcription factor of the TGF-_1 pathway that has been implicated in the regulation of proliferation and migration of different cell types. Moreover, we recently showed that SMAD3 is epigenetically repressed in SCC-TAFs compared to ADC-TAFs owing to an excessive exposure to cigarette smoke particles, which elicited a compensatory increase in its closely related homolog SMAD2 in SCC-TAFs. However, it remains unknown whether the differential SMAD2/3 expression between ADC and SCC-TAFs contributes to the larger accumulation of TAFs in ADC. To address this question, we knocked-down SMAD2 or SMAD3 in control pulmonary fibroblasts by shRNA and used them as ADC-like or SCC-like models. By assessing proliferation, we found that shSMAD2 fibroblasts exhibited a significantly lower number density in basal conditions compared to shSMAD3 fibroblasts, later confirmed in TAFs. In the presence of TGF-_1, number density increased and attained similar values in shSMAD2 and shSMAD3 fibroblasts as well as in ADC and SCC-TAFs. To assess fibroblast migration, we used a microfluidic device to quantify biophysical descriptors of protrusions and subsequent migration within a 3D collagen culture. Notably, both protrusions and migration descriptors were increased in basal conditions selectively in shSMAD2 fibroblasts, and TGF-_1 impaired migration. These results reveal that altered SMAD2/3 expression provide growth and migration advantages only in the absence of TGF-_1. These findings strongly support that the larger TAF accumulation in ADC occurs at early stages (under low TGF-_1) and is driven by the enhanced migration of ADC-TAFs due to their high SMAD3.

POSTER 38 presented by:

NAME: Annalisa Calò
GROUP: Nanoscale bioelectrical characterization
INSTITUTION: Universitat de Barcelona

Distinct fibroblasts phenotypes from lung cancer revealed by force-volume AFM

Fibroblast cells evolve from a quiescent, non-metabolically active state to an active state during cancer aggression. This state is characterized by altered protein expression, cytoskeleton remodeling and enhanced production of extracellular matrix (ECM) components. The identification of fibroblasts phenotypes associated to different types of cancer can help understanding the molecular mechanism that gives rise to the disease and can constitute the base for developing therapeutic strategies. In this work we present the AFM operating in force volume mode (QI mode) to identify fibroblasts phenotypes associated to two different cancers of the lung, the adenocarcinoma (ADC) and the squamous cell lung carcinoma (SCC). The two phenotypes are clearly distinguishable from the mechanical parameters (Young'ss modulus, stiffness, out of contact baseline), which are obtained on extended fibroblast monolayers in a label-free way. To obtain statistically robust datasets (tens of thousands points per patient), we developed a measurements protocol to test millimeter-size areas of the samples with in affordable time scales, using colloidal probes. Measurement of one patient sample takes less than 1 hour; in this way, AFM measurements and data analysis of 2-3 patient samples can be performed in one day. The measurements protocol is versatile and can be applied to different type of samples, from live cells to tissue sections, when micrometer size resolution is required. In this way, functional AFM maps can be made readily available to the scientific community, also for correlation to other techniques (optical microscopy images, histological sections etc).

POSTER 39 presented by:

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

Mechanics of crypt-villus boundary formation and differentiation in intestinal organoids

The intestinal epithelium is a monolayer of cells that covers the surface of the gut. It protects against pathogens, absorbs nutrients and secretes hormones and other molecules. This monolayer is folded into finger-like protrusions called villi and invaginations called crypts. Essential features of the intestinal epithelium such as folding of the crypt, spatial distribution of different cell types, and cellular movements from crypt to villus-like domains can be captured *in vitro* in intestinal organoids. By direct mechanical measurements of mouse intestinal organoids grown on soft hydrogels, we show that tightly regulated cell-ECM and cell-cell forces define mechanical and functional compartments within the epithelium (i.e the stem cell niche, the transit amplifying zone and the villus-like region). The transit amplifying region appears as a mechanical boundary of basally constricted cells that separate undifferentiated cells at the stem cell niche from differentiated cells at the villus-like region. Of note, crypt-villus boundary formation and mechano-functional compartmentalization of the tissue co-evolve with cell fate specification. We thus hypothesize that the transit amplifying region may serve as a hub of mechanically-induced cell differentiation. We are currently addressing this question by perturbating crypt villus-boundary formation through genetic (i.e. EphB2/3 KO organoids) and biochemical means (i.e. cytoskeletal drug treatments).

POSTER 40 presented by:

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

Harnessing active viscoelasticity to generate epithelial folds

Epithelial sheets 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. To generate and maintain these structures, epithelia must undergo complex 3D deformations across length and time scales. How epithelial shape arises from active stresses, viscoelasticity and luminal pressure remains poorly understood. To address this question, we developed a microfluidic chip and a computational framework to engineer 3D epithelial tissues with controlled shape and pressure. In the setup, an epithelial monolayer is grown on a porous surface with circular low adhesion zones. On applying hydrostatic pressure, the monolayer delaminates into a spherical cap from the circular zone. This simple shape allows us to calculate epithelial tension using Laplace'ss law. Through this approach, we subject the monolayer to a range of lumen pressures at different rates and hence probe the relation between strain and tension in different regimes, while computationally tracking actin dynamics and their mechanical effect at the tissue scale. Slow pressure changes relative to the actin dynamics allow the tissue to accommodate large strain variations. However, under sudden pressure reductions, the tissue develops buckling patterns and folds with different degrees of symmetry-breaking to store excess tissue area. These insights allow us to pattern epithelial folds by rationally directed buckling. Our study establishes a new approach for engineering epithelial morphogenetic events.

POSTER 41 presented by:

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

Mechanical phenotyping of colorectal cancer patient derived organoids based on LGR5 expression

Colorectal cancer (CRC) tumors are composed by heterogeneous cell populations comprising differentiated cells and a small pool of cancer stem cells (CSCs). Despite extensive investigation on the role of cancer stem cells in cancer progression and metastasis formation, whether cancer cell differentiation states are linked to specific mechanical features remains unknown. Adopting a bottom-up approach, we performed a broad biophysical characterization of CRC patient derived organoids (PDOs), engineered to fluorescently label cells expressing LGR5, a well-established marker for CSCs. We show that CRC cells differentiation states are associated with distinct biomechanical phenotypes at the single cell level. Cells expressing high levels of LGR5 differ from differentiated-like cancer cells in shape, traction force distribution, cell stiffness and amoeboid response to confinement. These differences translate to distinct migratory and morphological phenotypes at a cluster level with LGR5 expression being negatively correlated with clusters roundness and migration speed. At higher complexity levels, such as interactions with endothelial cells, clusters expressing more LGR5 show higher attachment rate to the endothelium compared to differentiated-like clusters. Based on these findings relating distinct mechanical phenotypes to LGR5 expression, we speculate that mechanical adaptability coupled with cancer plasticity may be an indispensable mechanism for cancer progression.

POSTER 42 presented by:

NAME: Jose Antonio del Rio
GROUP: Molecular and cellular neurobiotechnology
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Involvement of mechanical cues in the migration of Cajal-Retzius cells in the marginal zone at early stages of cortical development

Emerging evidence points to coordinated action of chemical and mechanical cues during brain development. At early stages of neocortical development, angiogenic factors and chemokines such as CXCL12, ephrins, and semaphorins assume crucial roles in orchestrating neuronal migration and axon elongation of postmitotic neurons. Here we explore the intrinsic mechanical properties of the developing marginal zone of the pallium in the migratory pathways and brain distribution of the pioneer Cajal-Retzius cells. These pioneer neurons are generated in several proliferative regions in the developing brain (e.g., the cortical hem and the pallial subpallial boundary) and migrate tangentially in the preplate/marginal zone covering the upper portion of the neocortex. These cells play crucial roles in correct neocortical layer formation by secreting several molecules such as Reelin. Our results indicate that the motogenic properties of Cajal-Retzius cells and their perinatal distribution in the marginal zone are also modulated by both chemical and mechanical factors, by the specific mechanical properties of Cajal-Retzius cells, and by the differential stiffness of the migratory routes. Indeed, cells originating in the cortical hem display higher migratory capacities than those generated in the pallial subpallial boundary which may be involved in the differential distribution of these cells in the dorsal-lateral axis in the developing marginal zone.

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POSTER 43 presented by:

NAME: Natalia Diaz Valdivia
GROUP: Associated Researcher
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

SMAD3 signaling in tumor associated fibroblasts promotes early cancer cell invasion in 3D cultures and *in vivo* in lung adenocarcinoma

Lung cancer is the leading cause of cancer death worldwide, lung adenocarcinoma (ADC) and squamous cell carcinoma (SCC) are their most common histologic subtypes. Although both are epithelial in origin, the fibrotic tumor microenvironment rich in tumor-associated fibroblasts (TAFs) plays a critical role in all steps of tumor progression. There is clinical evidence that ADC tumors tend to disseminate earlier than SCC tumors, although the underlying mechanisms remain unknown. We recently reported that transcription factor SMAD3 was epigenetically repressed in SCC-TAFs compared to ADC-TAFs, which elicited a lower SMAD3 expression and activity that was compensated by a larger expression and activity of SMAD2 selectively in SCC-TAFs. Since there is growing evidence that SMAD2/3 regulate cell migration, we analyzed the impact of altered SMAD2/3 expression in fibroblasts in their ability to lead cancer cell invasion. For this purpose, we knocked-down either SMAD2 or SMAD3 in primary pulmonary fibroblasts, and used them as ADC-like or SCC-like fibroblast models, respectively. To study collective cell invasion, we formed 3D tumor spheroids by mixing ADC cancer cells and TAFs within a mixture of collagen and basement membrane, what revealed more invasive events in tumor spheroids containing shSMAD2 (ADC-like) fibroblasts compared to shSMAD3 (SCC-like) fibroblasts. We coinjected ADC cells with fibroblasts into immunodeficient mice and monitored tumor growth, what showed that tumors bearing shSMAD2 fibroblasts exhibited larger tumor growth and developed more invasive structures than tumors bearing shSMAD3 fibroblasts. Mechanistically, shSMAD2 fibroblasts exhibited larger expression of N-cadherin, which we previously implicated in collective cancer cell invasion led by TAFs through enhanced force transmission. Our results reveal that SMAD3 in fibroblasts drives tumor growth and invasion of ADC cells, which may contribute to the early dissemination that is frequently observed in the clinic selectively in ADC.

POSTER 44 presented by:

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

A new system revels that single epithelial cells can exert pushing forces on their environment

From tiny sperm cells to star shaped neurons, cell size and shape is intimately linked to function. Recently, cell morphology has been correlated to the response to mechanical signals, with cell spreading being associated with mechanical force transmission by cells. Though interesting, most studies use 2 dimensional (2D) systems and thus do not recapitulate to the full extent 3 dimensional (3D) cell shape. This holds particularly for epithelial cells due to the importance of mechanical homeostasis inside the epithelium and its role in wound healing. In this work, we have developed a structured hydrogel system that enables us to measure, in 3D, the amount of forces exerted by a single-cell of controlled morphology. With it, we report a novel phenomenon in which breast epithelial cells exert pushing forces on their environment, and not only pulling forces as previously described. Moreover, we demonstrate that the shift from pulling to pushing is correlated with a decrease in cell volume. Whereas the actin cytoskeleton plays a role in both behaviors, myosin II is only implicated in contractile (pulling) activity. More generally, this raises the question of the importance of such a phenomenon in an epithelium, where the cell volume and mechanical homeostasis need to be constantly maintained.

POSTER 45 presented by:

NAME: Amélie Godeau
GROUP: Bioengineering in Reproductive Health
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Mechanics Of Mouse and Human Embryo Implantation

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 exerting 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 mouse embryos, are also mechanosensitive reacting to external force cues such as spheroids or collagen pulling by a microneedle leading to an alignment of the proximal-distal embryo axis. We conclude that mechanical forces play a key role, both in guiding the invasion of the extracellular matrix during implantation but also in the symmetry breaking and axis formation of the embryo.

POSTER 46 presented by:

NAME: Miguel González-Martín
GROUP: Cellular and molecular mechanobiology
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Designing synthetic mechanosensitive molecules for the mechanical control of transcription

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 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 transcription factors with nucleocytoplasmic transport properties sensitive to force-driven nuclear deformations. By adjusting the mechanical properties and tuning the nuclear localization signals of these transcription factors we will be able to alter the transcription levels of a desired gene of interest with force. 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 the application simplicity. In fact, it will allow the implementation of mechano-sensitivity in transcription factors within synthetic gene circuits. Applications include creating reporters, inverting mechanical- associated phenotypes, or creating of a new set of tools for future biotechnology research in 3D structures, where forces play a major role.

POSTER 47 presented by:

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

Development of a sensor to study mechanotransduction in the nucleus

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. The sensor was tested in single cell Mouse Embrionary Fibroblasts, and now we are carrying a multicellular approach with epithelial cell monolayers to understand how cells in monolayers are affected by forces in the nucleus. For this, we compute sensor ratio, cell density, nuclear shape parameters, nuclear size, and also inhibit specific force generators in the cell with specific molecular manipulations.

POSTER 48 presented by:

NAME: Alejandro Llorente Álvarez
GROUP: Cellular and molecular mechanobiology
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Characterisation of the immunosuppressive role of activated fibroblasts in non-small cell lung cancer

Lung cancer is the leading cause of cancer death, with an 18% survival at 5 years of diagnosis. Recent advances in immunotherapy (such as anti-PD-L1 therapy) have shown improved survival, showing long-term therapeutic responses in selected patients in advanced stages. However, there are patients who showed poor response or acquire resistance, suggesting alternative immunosuppressive mechanisms other than PD-L1. It is known that activated tumor-associated fibroblasts (TAFs) play a key immunosuppressive role, including both the secretion of immunosuppressive factors (IF) that reduce the activity and recruitment of effector T cells and favor the recruitment of immunosuppressive cells (like macrophages-M2) as well as the induction of PD-L1 expression. In addition, activated TAFs generate a stiff microenvironment, impairing the infiltration of the immune system and the drug distribution, favoring hypoxia, that promotes the recruitment of Tregs and promotes PD-L1 expression in cancer cells. Although, TAF-dependent immunosuppressive factors in non-small cell lung cancer (NSCLC) are poorly defined. We found in the literature at least 27 IF that can be secreted by fibroblast and identified 16 presents in NSCLC patient-derived TAFs by RNAseq and qRT-PCR. Among these factors, 4 are regulated by TGF-_1 and 3 that are only present in TAF but not in control fibroblasts. This data suggests that TAFs contributed to immunosuppression in NSCLC, and the use of antifibrotic drugs could attenuate this effect, improving the immunotherapy outcome.

POSTER 49 presented by:

NAME: Aishwarya MK
GROUP: Nanobioengineering
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Development of splenic chip model for the RBC mechanical properties evaluation

Rare hereditary haemolytic anemias(RHHA) affect millions of people every year across the world irrespective of age, sex, and race. Spleen has a key role in RHHA, which consists of splenon acting as filtering unit of the blood by prematurely removing the deformed or diseased RBCs. Even though there are many diagnostic techniques available for the analysis of these disorders, they are not very effcetive and there is need for advanced diagnostic tools.Recently organ on chip models are explored to study the meachnism of these RHHA which helps in the RBCs shape analysis but not cell-cell intereactions. Our main objective is to mimic the physiological splenon filtering system for studying the disease mechanism through the evaluation of the RBCs interaction with spleen cells and RBCs shape analysis. Here we developed a spleenic chip model that mimics the filtering part of the spleen which consists of microconstrictions representing the inter endithelial slits and pillar matrix for the fibrous netwok in the splenon. the device is made with different kinds of fabrication techniques such as photolithography; backside illumination photolithography and soft lithography and are characterised by profilometer for measuring the thickness and optical microscope for device structure analysis and SEM for surface topography. Before fabrication we have done the COMSOL simulations which helps in the optimization of the device design. The developed device is co-cultured with spleen cells and the RBCs are passed through the device and the interactions were characterised by optical microscope. In conclusion the spleen on a chip device can be used for studying RBC mechanical properties which helps as diagnostic device for the RHHA.

POSTER 50 presented by:

NAME: Marc Molina Jordán
GROUP: Cellular and molecular mechanobiology
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Exploring how force transmission to nuclear pore complexes affects nucleocytoplasmic transport

Transport of molecules between the nucleus and the cytoplasm is one of the most tightly controlled processes in cells. Recently, the application of mechanical force to the nucleus has been shown to regulate nuclear transport. Nuclear pore complexes (NPCs), as the gateways to the nucleus, stand at the center of this process. However, the mechano-molecular link of force transmission to the NPC remains unclear. Through super-resolution imaging, we show that nuclei submitted to force have NPCs with increased diameters. Further, knocking down two key structural NPC proteins, nucleoporins 153 (NUP153) and 155 (NUP155), results in a decrease in nuclear accumulation of the transcriptional regulator (TR) YAP/TAZ, which is known to be regulated by nucleocytoplasmic transport. This evidence suggests that these proteins may regulate the structural integrity of NPCs under force, or even be involved in the transmission of force to the NPC. To further explore this hypothesis, we are carrying out experiments to measure diffusion across NPCs in the different conditions, and to perturb force transmission between nuclei and the cytoskeleton.

POSTER 51 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

Organ formation during development is a complex, yet beautifully orchestrated process. Embryonic cells have a unique capacity to self-organize within the forming tissue, where morphogenetic movements facilitate tissue organization and subsequent organ formation. In kidney organogenesis, the mature organ arises from crucial reciprocal interactions between the ureteric bud (UB) and metanephric mesenchyme (MM), which give rise to the collecting duct and nephron, respectively. The development of mature nephrons during kidney organogenesis is a dynamic process so far studied via *in vivo* models. Accumulative findings in mice have shown that the MM undergoes mesenchymal to epithelial transition (MET), giving rise to epithelial renal vesicles (RVs) that further undergo structural changes and shift towards comma-shaped and s-shaped bodies (CSBs/SSBs), which eventually develop into nephron like structures. Those studies have helped identify Wnt/b-catenin and Notch signaling pathways as key players in nephron patterning and segmentation (proximal, medial, distal segments). At the same time, tissue morphogenesis is largely a biomechanical process, resulting from constant movements of cells, changes in forms of developing segments, and forces generated therein. The biomechanical dynamics occurring during RV emergence and further nephron patterning are yet to be explored in the human context in real-time. Whether these processes are interconnected with mechanical signals remains an open question in the field. The answer may have an important impact on understanding nephron formation, and conversely, disease-related phenotypes due to mutations in genes orchestrating RV patterning and segmentation, as seen in congenital defects of the kidney and the urinary tract (CAKUT disease). Here, we aim to use human pluripotent stem cell (hPSCs) derived kidney organoids to understand 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 polyacrylamide (PAA) and PDMS hydrogels with controlled rigidities (mimicking embryonic microenvironment) in a 2D culture system. PAA and PDMS hydrogels between 3 kPa (soft) and 60 kPa (rigid) are generated by adapting the compositional ratio of acrylamide to bis-acrylamide, or PDMS components respectively, and are further functionalized and decorated with fibronectin. Using this system, we have started to spatiotemporally characterize early steps of nephrogenesis by immunofluorescence and confocal analysis, time-lapse imaging, and traction force microscopy (TFM). These analyses are conducted during RV emergence before proximal-distal RV polarization and the formation of the nephron-like segments. The current techniques will permit quantitative and qualitative observations of multicellular behaviors at key stages of 2D renal differentiation. Furthermore, this system will allow us to spatiotemporally map cell-cell and cell-ECM forces and evaluate their evolution throughout renal fate specification. The final aim is to decouple mechano-related processes sustaining nephron formation from classical biochemical signaling.

POSTER 52 presented by:

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

Inhibiting mechanotransduction as a novel approach for oncology therapy

Most solid tumors show an increase in tissue stiffness, which drives tumor progression. While this fact has clear clinical implications, currently, there are no compounds or drugs available to target mechanotransduction. Our lab has demonstrated that tissue stiffness triggers unfolding of the target protein and induce an interaction with its binding partner. This interaction results in the nuclear translocation of a mechanosensitive transcription factor that has been implicated in the progression of many solid tumors. Therefore, blocking the interaction between the target and its binding partner has a major potential as a therapeutic approach in several solid cancer types. Based on this study, a novel approach to inhibit mechanotransduction is envisioned. Our aim is to identify small molecules that inhibit the stiffness-induced unfolding of the target protein, thereby blocking interaction with its partner. The hypothesis is that compounds that stabilize the target protein in turn reduce its unfolding and hence inhibit the interaction. The impact of this in cellular context is the inhibition of stiffness-mediated nuclear translocation of mechanosensitive transcription factors, and hence decrease in cell proliferation. We have developed an assay to conduct High-throughput screening of diverse chemical libraries to identify hits molecules that bind to and stabilize the target. Further, a cascade of assays is designed to validate and delineate the mechanism of action of the identified hit molecules. The outcome of this project will be a first-in-class mechanoinhibitor, drug-like compound with therapeutic applications in several cancer types and other pathologies wherein mechanotransduction is implicated. Additionally, the compound may provide a useful tool for mechanobiology research.

POSTER 53 presented by:

NAME: Isabela Corina Santos Fortunato
GROUP: Integrative cell and tissue dynamics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Mechanisms controlling cell migration during haptotaxis and reverse-haptotaxis

The ability of cells to perform directed migration is an essential mechanism involved in important tissue processes such as morphogenesis, immune function, metastasis, and fibrosis. Directed cell migration is often triggered by spatial gradients in the cellular environment. These extracellular cues are mainly perceived by cells through a protein complex called focal adhesions (FAs). Conformational changes of key FAs proteins are force-induced and mediate the mechanosensing process by its linkage to the actomyosin cytoskeleton. After sensing these gradients, cells exhibit a subcellular organization that leads to a well-defined front-to-rear polarization of the cytoskeletal structures.Haptotaxis, - the ability of cells to follow gradients of substrate-bound ligands -, has been described as one of the essential key promotors of directed migration in physiologic and pathologic processes. However, the molecular mechanisms underlying haptotatic migration are still unclear. To better understand haptotaxis, 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. Cells lost this initial haptotatic response under inhibition of ROCK (Y-27632) but not myosin II (blebbistatin). Remarkably, we also observed that after the initial polarization, cells maintained their directionality even if they were submitted to a negative protein gradient (reverse-haptotaxis). Thus, our results indicate that, contrarily to what has been described in other spatial gradients (e.g., stiffness gradients), haptotaxis is myosin II independent. Furthermore, we show that once the cell is polarized and forced to move down the gradient, there is an adaptation of the actin polymerization and actomyosin contractility velocities that allow the cell to move in reverse-haptotaxis.

POSTER 54 presented by:

NAME: Ignacio Viciano Gonzalo
GROUP: Cellular and molecular mechanobiology
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

In silico development of small molecules to block mechanotransduction for pancreatic cancer therapy

Mechanobiology is a discipline that studies how cells and tissues respond to mechanical factors such as tissue stiffness, and their implications in diseases such as cancer. Our research group discovered few years ago that when the tissue is rigid, the Talin protein unfolds thus exposing several cryptic binding sites to the Vinculin protein. The Talin-Vinculin binding leads to 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 tried to unveil from the point of view of molecular modeling the binding mechanism of these two proteins. Furthermore, we have carried out a computer-aided drug design project with the aim of discovering small molecules that inhibit the interaction of these two proteins. These molecules could be a first approach to create potential new drugs for the treatment of Pancreatic cancer.

POSTER 55 presented by:

NAME: Srivatsava Viswanadha Venkata Naga Sai
GROUP: Cellular and molecular mechanobiology
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Characterizing the role of mechanotransduction in mouse embryonic stem cells

Mouse Embryonic Stem Cells (mESCs) possess ground/naïve state pluripotency when grown in defined N2B27 media supplemented with two inhibitors (2i), for Erk and GSK3_. 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). Although, Laminin was found to be the pivotal ECM ligand for pluripotency dissolution, 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. Finally, attenuating the functionality of said axis by contractility inhibition or by targeting LINC complex to abrogate nuclear - cytoskeleton coupling altered naïve state exit kinetics.

POSTER 56 presented by:

NAME: Joana Admella Pedrico
GROUP: Bacterial infections: antimicrobial therapies
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

A straightforward method for isolation and cultivation of Galleria mellonella hemocytes

Galleria mellonella is an alternative infection animal model. It presents a wide range of advantages as their maintenance and rearing are both easy and inexpensive. Being more ethically accepted than other models, it has a convenient size for manipulation and its immune system has multiple similarities with the one found in mammals. Hemocytes are immune cells that help encapsulate and eliminate pathogens and foreign particles. All of this makes this insect a very promising model. However, cultivating G. mellonella hemocytes *in vitro* is not straightforward, and it is challenging for its several difficulties. Here we present a protocol with different methodological optimizations for the establishment and maintenance of a G. mellonella hemocytes primary culture. These improvements open the door to studying both easily and quickly the toxicity of nanoparticles and the interactions of particles and materials in an *in vivo* environment.

This study was partially supported by grants RTI2018-098573-B-I00 and PID2021-125801OB-100 funded by MCIN/AEI/ 10.13039/501100011033 and "ERDF A way of making Europe", the CERCA programme and AGAUR-Generalitat de Catalunya (2017SGR-1079), the European Regional Development Fund (FEDER), Catalan Cystic Fibrosis association and Obra Social "La Caixa". JA is thankful to the Generalitat de Catalunya for its financial support through the FI program (2021FI_B 00118).

POSTER 57 presented by:

NAME: Lara Victoria Aiassa
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Phenotypic targeting of different macrophages differentiation states

Mononuclear phagocytes are fundamental for the resolution of inflammation by clearing apoptotic material at inflamed sites and undertaking pro-resolution signalling [1]. Macrophages exhibit an extraordinary environmental stimuli responsive plasticity arising from a broad spectrum of phenotypes conventionally classified as classically activated pro-inflammatory M1 or activated anti-inflammatory M2 macrophages. The different macrophage phenotype populations are in optimal balance, promoting tissue homeostasis [2]. However, alteration of this equilibrium is translated into disease onset. Evidence suggests that ageing is associated with an overt inflammatory phenotype and failure to engage pro-resolving pathways that contribute to persistent chronic inflammation [1,3,4].Modulating macrophage phenotype and function remains a key therapeutic goal for treating several diseases with macrophages as main effector cells like in chronic inflammation, cancer and infectious diseases like Tuberculosis, among others. To address this, we propose the design of super-selective nanoparticles functionalised to target specific macrophage differentiation states. A detailed study of macrophage phenotype has been performed to identify the characteristic receptor expression pattern and the glycans expression profile to determine the receptor expression fingerprint for each macrophage polarisation state. We have selected a set of ligands to design the optimal multiplexed-multivalent system capable of discriminating between macrophage states creating on-off association profiles, and achieving phenotypic targeting.

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POSTER 58 presented by:

NAME: Julia Alcacer Almansa
GROUP: Bacterial infections: antimicrobial therapies
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Antibiotic susceptibility of Pseudomonas aeruginosa and Burkholderia cenocepacia in planktonic cultures and multispecies biofilms

Pseudomonas aeruginosa and Burkholderia cenocepacia are two multidrug-resistant opportunistic pathogens often found in the form of biofilms in the lungs of patients with chronic obstructive pulmonary diseases. Multispecies biofilms are aggregates consisting of multiple interwoven or adjacent microorganisms, allowing potential interspecies interactions. Biofilm-forming bacteria show survival-promoting phenotypic traits, including drug resistance mechanisms that complicate their eradication. Specific compositions of bacteria in multispecies biofilms also play a crucial role in the efficacy of antimicrobial therapies, and particular bacterial distributions can increase their tolerance towards them. Understanding the role of P. aeruginosa and B. cenocepacia in the antibiotic resistance profile of their multispecies biofilm is crucial to improving the management of those infections. In this context, the present work aims at establishing a comparison between the bactericidal effects of three existing antibiotics in planktonic and sessile co-cultures and single-species cultures of P. aeruginosa and B. cenocepacia. An increase in the minimal inhibitory concentration was observed when both bacteria are forming single- or multispecies biofilms compared to when bacteria are growing in planktonic conditions. In addition, the effects of antibiotic treatments differed depending on whether bacteria were growing in single- or multispecies biofilms.

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POSTER 59 presented by:

NAME: Ana Alves
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Synergic approach to the treatment of glioblastoma through a novel xanthone-polymersome system

Globally, about 189,000 people die every year as a result of any kind of brain cancer, being glioblastoma (GBM) the most common and aggressive form. Chemotherapy is difficult due to the heterogeneity of brain cancer and great efficacy of Blood Brain Barrier (BBB) making drug absorption into brain very difficult.Polymersomes (PMs) are artificial vesicles enclosing an aqueous cavity, resulting from the self-assembly of amphiphilic copolymers.These sequences of polymers depend on the number of polymer chains, and originate versatile structures that can encapsulate hydrophilic or hydrophobic drugs. PMs vary in charge and dimension, are biocompatible and biodegradable, have demonstrated low *in vivo* toxicity, and showed better physical and chemical properties than liposomes.The main of this study is to develop a dual targeted delivery system based on di-block polymersomes (PEG-PLA) able to cross the BBB and deliver a synthetic xanthone with proven antitumor activity, at the site of the tumor. The first barrier to overcome is the BBB, this will be achieved by the surface modification of the polymersomes with transferrin, which receptors are known to be overexpressed on the surface of brain capillary endothelial cells, a major part of BBB. Additionally the glioma cells will also be targeted by the use of hyaluronic acid, to address the highly expressed CD44 receptor on glioma cell subpopulations.

POSTER 60 presented by:

NAME: Conrado Aparicio
GROUP: Associated Researcher
INSTITUTION: UIC Barcelona - Universitat International de Catalunya

Basement membrane-derived peptides nanocoatings to promote percutaneous device soft tissue attachment and prevent infection

Teeth are long-lasting percutaneous organs that feature soft tissue attachment through adhesive structures, hemidesmosomes, in the junctional epithelium basement membrane adjacent to teeth. This soft tissue attachment prevents bacterial infection of the tooth despite the harsh microbial composition of the oral cavity. Conversely, millions of percutaneous devices (catheters, dental, and orthopedic implants) fail from infection yearly. Infection prevention strategies have failed in generating durable soft tissue adhesion - like that seen with the tooth - to prevent biofilm colonization at the tissue-device interface. Here, Inspired by the impervious natural attachment of the junctional epithelium to teeth, we synthesized four cell adhesion peptide (CAPs) nanocoatings, derived from basement membranes, to promote percutaneous device soft tissue attachment. The two leading nanocoatings upregulated integrin-mediated hemidesmosomes, selectively increased keratinocyte proliferation compared to fibroblasts, which cannot form hemidesmosomes, and expression of junctional epithelium adhesive markers. CAP nanocoatings displayed marked durability under simulated clinical conditions and the top performer CAP nanocoating was validated in a percutaneous implant murine model. Basement membrane CAP nanocoatings, inspired by the tooth and junctional epithelium, may provide an alternative antiinfective strategy for percutaneous devices to mitigate the worldwide threat of antimicrobial resistance.

POSTER 61 presented by:

NAME: Betsy Verónica Arévalo Jaimes
GROUP: Bacterial infections: antimicrobial therapies
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Influence of culture media in Candida parapsilosis *in vitro* biofilms

Candida parapsilosis is responsible for about 16% of Candida infections, the second most frequent in candidemia cases and a significant problem in neonates. The increased incidence of C. parapsilosis is associated with their ability to form biofilms even in high-glucose environments. However, its incapability to form true hyphae ends in smaller and less complex biofilms than those generated by Candida albicans. This particularity makes C. parapsilosis biofilm *in vitro* studies of great importance to understand and develop preventive and therapeutic strategies against this fungal pathogen. Nevertheless, there is no scientific consensus regarding the conditions employed for biofilm development. The cultivation media in Candida spp. has a profound effect on the metabolic adaptations of the yeast, reflected in morphology changes and, consequently, in the characteristics of the biofilm. For that reason, we evaluated the influence of four different culture media on the morphology, structure, and biomass of C. parapsilosis biofilms. Moreover, the effect of oxygen availability and flow conditions were also explored. Our results evidence that pseudohyphae morphogenesis in C. parapsilosis is directly influenced by the cultivation media, with RPMI displaying a higher transition to this form. In addition, the quantity of pseudohyphae in the biofilm increase when it is formed with less oxygen availability and under shear stress forces. Regarding biomass, the high glucose content in YPD media allowed the highest growth of C. parapsilosis biofilms. In conclusion, the election of cultivation media for *in vitro* biofilm studies should be done according to the aim and implications of the study.

This study was partially supported by grants RTI2018-098573-B-I00 and PID2021-125801OB-100 funded by MCIN/AEI/10.13039/501100011033 and "ERDF A way of making Europe", the CERCA program and AGAUR-Generalitat de Catalunya (2017SGR-1079), the European Regional Development Fund (FEDER), Catalan Cystic Fibrosis association and "La Caixa" Foundation. Additional resources were obtained by an INPhINIT Fellowship (LCF/BQ/DI20/11780040) from "La Caixa" Foundation.

POSTER 62 presented by:

NAME: Yunuen Avalos-Padilla
GROUP: Nanomalaria
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Aptamers targeting Plasmodium falciparum ESCRT-III proteins as a potential antiplasmodial tool

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, cheap design, 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 fixed Plasmodium-infected RBCs. Furthermore, purified single DNA aptamer sequences have been tested for their antiplasmodial activity, showing a mild effect against the parasite'ss viability and specific binding towards Plasmodium late stages.

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POSTER 63 presented by:

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

Evaluating the collective dispersion of magnetic-enzymatic nanomotors

Many treatments are based on the systemic administration of high amounts of therapeutic drugs, which leads to side effects and limited accumulation at the target side. Therefore, methods to efficiently administer, penetrate and locally release the drugs such as smart nanoparticles (NPs) for precision medicine are highly needed. For this, NPs with self-propelling properties, which are called nanomotors, have been proposed as drug delivery systems able to overcome these limitations. However, the fundamental understanding of the collective movement i.e. swarming behavior of these nanomotors is still lacking. And many nanomotor approaches are either showing high velocities or directional steering abilities, but not both at once. Here, we present nanomotors with dual functionalities: they can be enzymatically powered and magnetically steered. These properties are being investigated as a novel strategy to deliver drugs with high precision. The movement behavior was analyzed using image analysis tools (Matlab) of the recorded videos of swarming nanomotors. This allows for a better understanding and quantification of the collective dispersion under different experimental conditions, such as the concentration of fuel or magnetic field configuration. To apply different magnetic field configurations, a permanent magnet array, a so called Halbach-ring, has been developed, able to apply homogeneous and gradient magnetic fields.The results show swarming behaviors in the presence of the fuel for nanomotors due to their enzymatic activation, as well as directionality due to the application of magnetic fields. The conducted image analysis gains an insight into these combined movement behaviors.

POSTER 64 presented by:

NAME: Valentino Barbieri
GROUP: Molecular bionics
INSTITUTION: UCL

Hybrid Au-polymersomes as photoactivated nanomedical agents

We engineered hybrid Au-polymersomes via a facile in situ templated Au reduction in the membrane of pH-sensitive block copolymer polymer vesicles. Hundreds of monodisperse 2 nm AuNPs were loaded into each vesicle without negatively affecting the colloidal stability of the system. The Au-polymersomes display an enhanced absorbance of visible photons which stimulates a macroscopically detectable thermoplasmonic heating of the sample in response to external illumination. Furthermore, we tested the application of our Au-polymersomes for photothermal therapy. *In vitro* experiments on human glioblastoma T98G cells, showed an over 10-fold increased uptake of Au from our systems compared to benchmark citrate-stabilized AuNPs. We believe this quick internalisation to result from the highly selective targeting of specific scavenger receptors by the phosphorylcholine moiety in our polymer. After the uptake, we exposed the cells to a low intensity scanning laser in live imaging assays, in which the death of cancer cell treated with Au-polymersomes was observed at a significantly higher rate compared to both untreated cells and cells treated with citrate-AuNPs. Cell blebbing events and the loss of the structural order of actin filaments in the cytoskeleton indicate an apoptotic death pathway. No increase in reactive oxygen species was detected, further confirming the photothermal origin of the phenomenon.We conclude that embedding AuNPs in hybrid vesicles can augment the efficiency and range of application of their plasmonic properties in biologically relevant scenarios by maximising intracellular delivery.

POSTER 65 presented by:

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

Modulating misfolded proteins transcytosis in the blood-brain barrier

One main hallmark of neurodegenerative diseases, such as amyloidosis, synucleinopathies, and tauopathies, is protein aggregation, an uncontrolled accumulation of misfolded proteins in the central nervous system (CNS). Misfolded protein aggregates trigger pathological pathways that induce cell dysfunction and tissue damage, heading to the disease. The blood-brain barrier (BBB) plays a main role in regulating the clearance of misfolded proteins from the CNS, which diminishes pathologies such as Alzheimer'ss disease. One of the receptors involved in this process is the low-density lipoprotein receptor-related protein 1 (LRP-1), which mediates the shuttling of amyloid-_ (A_) across the BBB. A recent study demonstrated that the LRP1-mediated transcytosis of A_s happens through the formation of tubular structures stabilized by the Bin/Amphiphysin/Rvs (BAR) protein syndapin-2 (Tian et al., Sci. Adv. 2020; 6, eabc4397 and Leite et al. Brain Comm., 2022, 4, fcac039). To stimulate such a physiological process, we formulate functionalised polymeric nanoparticles whose avidity, based on multiple ligand-receptor affinities, can reproduce what happens *in vivo*. To support our theory, we evaluate the expression of the main genes involved in the transcytosis, as well, as we deeply characterise our BBB *in vitro* model. The investigations herein are an important step to further assess the enhancement of the clearance of A_ from the CNS by using polymeric nanoparticles.

POSTER 66 presented by:

NAME: Inés Bouzón
GROUP: Nanomalaria
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Targeting protein aggregation in Plasmodium falciparum as a new antimalarial design strategy

The currently available arsenal of antimalarial drugs is insufficient to progress towards eradication of the disease, a scenario that is worsened by the rampant evolution of resistance by Plasmodium. Protein aggregation in malaria parasites is prominent in most of its stages in both the human and mosquito hosts. To test if inhibiting protein aggregation in the parasite might impair its development, we treated *in vitro* cultures with amyloid pan-inhibitors. One of these compounds, the bis-styrylpyridinium salt YAT2150, exhibits potent antimalarial activity with an *in vitro* IC50 of 90 nM in asexual blood stages. This drug is also active against the sexual and hepatic stages of Plasmodium. YAT2150 is a powerful inhibitor of the aggregation of the amyloid Beta peptide fragment 40 and it reduces the amyloid content and the amount of ubiquitinated proteins in P. falciparum cultures as well as the quantity of aggregative proteins detected with thioflavin T. Because resistance to all currently used antimalarial drugs is evolving rapidly in the parasite, the final objective of eradicating the disease has the discovery of new efficient antimalarials as a global health priority. In this regard, we observed that *in vitro* cultures of the parasite do not develop resistances against YAT2150, probably because this compound acts through new antiparasitic mechanisms not shared by other currently used drugs and targets many gene products. Thus, we propose YAT2150 as a promising compound for the post-artemisinin era.

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POSTER 67 presented by:

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

Mycobacteroides abscessus and Pseudomonas aeruginosa cooperate to evade immune system response

The incidence of infection by non-tuberculous mycobacteria, mainly Mycobacteroides abscessus, in cystic fibrosis patients and other chronic infectious diseases is increasing, translating into an acceleration in the decline of lung function. In most cases, M. abscessus is co-infecting with Pseudomonas aeruginosa, the most frequent pathogen in these diseases. However, it is not known how these two bacterial species interact when they are coinfecting. For this reason, this study analyzes the behavior of both species in some pathogenic relevant aspects: biofilm development, *in vitro* bronchial epithelial cells assays, and *in vivo* infections using the Galleria mellonella model. The results demonstrate both species' capabilities to form stable mixed biofilms and to inhibit single-biofilm progression reciprocally. Co-infections in bronchial epithelial cells are correlated with more significant proliferation cell inhibition. In G. mellonella, co-infections induce lower survival rates than individual infections.Interestingly, analyzing the immune response triggered by both bronchial epithelial cells and G. mellonella larvae, it is determined that P. aeruginosa induces overexpression of pro-inflammatory and melanization cascade responses, respectively. Contrary, co-infections prove an evident inhibition of the immune response in both models, which develops worse consequences for the host than the generated by the single P. aeruginosa infection. Overall, the presence of M. abscessus produces a decline in the immune responses that worsens the infection and compromise the host.

POSTER 68 presented by:

NAME: Natalia Castejon Savordelli
GROUP: Biomaterials for regenerative therapies
INSTITUTION: Universitat de Barcelona

Development and Rheological Characterization of smart stimuli responsive hydrogel for future application in periodontal regenerative medicine

Periodontal disease has been historically considered the most important global oral health burden, along with dental caries. Hydrogels represent an innovative approach not only in non-invasive fashion treatment but also in terms of sustained and controlled release of drugs. In this study, a novel injectable hydrogel was developed using chitosan, polyethylene glycol diacrylate, pluronic, and hyaluronate sodium for the continuous release of dexamethasone to exert pharmacological effects of anti-inflammation and tissue regeneration. As an active antiseptic Cetylpyridinium chloride offers a biofilm with a broad antibacterial spectrum. The formulations present optimal features in thermo- and photo responsiveness, rheological behaviour, biocompatibility, and biological activity to enhance the periodontium regeneration process. Dex-loaded micelles system was prepared using the thin-film hydration method. The amount of Dex-loaded was determined by measuring the UV absorbance. Rheological measurements were performed using a rheometer with a parallel plate geometry. Photopolymerization reaction was achieved and the biofilm was formed in 35 seg. The sol-gel transition occurred at 16,5 C. The effect of the oscillatory frequency range in both storage modulus and loss modulus increased with an increment in frequency. The results obtained in this study may serve as a benchmark for future investigations on the effect of hydrogel formulation by photopolymerization on regenerative medicine.

POSTER 69 presented by:

NAME: Giulia Cazzaniga
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Phagocyte-targeting polymersomes as intracellular delivery carriers for enzymatic inhibitors with antitubercular activity

Despite global efforts to develop innovative therapeutic approaches, tuberculosis (TB) remains one of the leading causes of mortality from a single infectious agent. We focused our attention on two protein targets known to be essential for the survival of Mycobacterium tuberculosis (Mtb) during infection: salicylate synthase I (MbtI), an enzyme that catalyses the first step in the biosynthesis of the iron-chelating siderophore mycobactin T, and Low-Molecular-Weight Phosphatase B (MptpB), which interferes with the host immune response. We have previously developed potent MbtI and MptpB inhibitors, which showed antimycobacterial activity at MIC concentrations as low as 30 μM. Now, we have encapsulated these drugs in PMPC-PDPA polymersomes (POs). Such a carrier allows us to (i) solubilise the hydrophobic drugs, (ii) target infected phagocytes via phenotypic association to scavenger receptors class B, (iii) efficiently deliver the cargo within the cell cytosol where most bacilli harbour, and (iv) effectively kill intracellular pathogens selectively. These POs combine the advantages of long-term stability with the potential to encapsulate a broad range of compounds. The POs were fully characterised by DLS, HPLC, TEM; their cytotoxicity was assessed against macrophage cell line (THP-1) and Primary Human Lung Fibroblasts (HLF). Future work includes evaluating the antimycobacterial activity of the drug-containing POs in Mtb-infected primary cells.

POSTER 70 presented by:

NAME: Claudia Codano
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Design and *in vitro* anti-inflammatory evaluation of PMPC-PPF-PMPC micelles

Neurodegenerative diseases such as Alzheimer'ss disease (AD) and Parkinson'ss disease (PD) progressively soar with the global ageing population. Underneath different pathogenic mechanisms, neuroinflammation results as a common trademark of neurodegenerative diseases. Much attention from scientific research is currently focused on finding new therapeutics that can re-establish the cytokine imbalance and therefore mitigate the neurodegeneration mechanisms.A few years ago, the US food and drug administration (FDA) approved the drug dimethyl fumarate (DMF), which activates the nuclear factor (erythroid-derived 2)-like 2 (NRF2) pathways, leading to anti-inflammatory effects. Our studies aim to transpose the anti-inflammatory properties of DMF to the nanoscale by designing poly-propylene fumarate (PPF)-based micelles which can release fumarate upon cellular uptake and metabolism. We will combine PPF with poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC), which we have proved targeting inflammatory cells such as macrophages and dendritic cells. The building blocks of the micelles were synthesised via reversible addition-fragmentation chain-transfer (RAFT) polymerisation, obtaining a triblock copolymer of PMPC and PPF (PMPC-PPF-PMPC). After the copolymer characterisation, PMPC-PPF-PMPC micelles were obtained by solvent-switch and characterised by Dynamic Light Scattering and Transmission Electron Microscopy. PMPC-PPF-PMPC micelles were then tested on differentiated monocytes and microglia cells to evaluate their cytotoxicity and anti-inflammatory properties.

POSTER 71 presented by:

NAME: Iris Cristina Da Luz Batalha
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Nanobiotics for the treatment of mycobacterial infections

Deaths caused by infections from antibiotic-resistant bacteria are expected to skyrocket over the next decades, with a staggering 10 million deaths per year projected for 2050. Treating infections by intracellular pathogens, such as M. tuberculosis, is 'a perfect storm's. The WHO revealed that while some 50 new antibiotics and 10 biologics are under development, only half of those target WHO-priority pathogens and the majority have very limited benefits when compared to existing antibiotics. Reformulating existent drugs in nanocarriers may help achieving enhanced efficacy and safety while reducing dose frequency, by providing temporal and localised control of drug exposure. In this work, we report the synthesis of dual-drug tuneable nanoparticle-based antibiotics, which showed increased bacterial killing efficacy in a zebrafish larval model of mycobacterial infection when compared to free drugs at the same concentration. In addition, nanoparticles were able to efficiently penetrate mycobacterial cords and granulomatous lesions - shielded regions of difficult access by free drugs, improving the therapeutic effect.

POSTER 72 presented by:

NAME: Maria del Moral
GROUP: Targeted therapeutics and nanodevices
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Role of PLGA copolymer ratio in the loading and release of therapeutic enzyme cargo

Poly(lactide-co-glycolide) (PLGA) nanoparticles (NPs) enhance the delivery of therapeutic enzymes for replacement therapy (ERT) of lysosomal storage disorders (LSD). Our previous studies optimized enzyme loading in biodegradable PLGA NPs and proved enhanced delivery in cellular and animal models. In this study, we focused on tuning of enzyme release using hyaluronidase (HAse), the enzyme deficient in an LSD called Mucopolysaccharidosis IX. PLGA NPs were synthesized using copolymers with either 50:50, 60:40, or 75:25 lactic:glycolic acid ratio, via double emulsion-solvent evaporation method. NPs were then incubated in simulating lysosomal conditions from 0.5 h to 4 weeks to analyze respective NP stability and enzyme release. 75:25 copolymer NPs were the most stable on lysosomal conditions, with no destabilization in the period tested, expected given their greater hydrophobicity and, thus, lower hydrolysis. Surprisingly, 50:50 NPs were more stable than 60:40 counterparts, which both destabilized by 2 weeks. This may be because the latter NPs were smaller, having a greater surface-to-volume ratio and smaller diameter, which may facilitate surface hydrolysis and water penetration for internal degradation. HAse release in lysosomal conditions was greater for 75:25 NPs, then 60:40 NPs, and finally 50:50 NPs. This was unexpected based on NP stability data and seemed to be rather related to copolymer hydrophobicity, from which hydrophilic HAse may escape prior to NP destabilization.

POSTER 73 presented by:

NAME: Aurora Dols Pérez
GROUP: Nanoscale bioelectrical characterization
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Effect of surface functionalization and loading on the mechanical properties of soft polymeric nanoparticles used as delivery systems

Recent studies have evidenced the importance of nanoparticle mechanics in their uptake and efficacy. Different strategies have been developed to tune NP mechanics but is still unknown the effect of loading or functionalization on the NP mechanical properties. The main drawback to study these factors is the difficulty to find a fabrication technique that allows to modify the inner part of the NP, to functionalize the surface, to change the composition, but obtaining comparable particle structures and sizes. In here, we performed a study of the effect of these parameters using Phase Inversion Composition method (PIC) to create NPs with similar composition, structure and sizes, and determine the effect of functionalization on the NP mechanics. Samples studied were PLGA NPs, PLGA NPs containing rhodamine 6G (PLGA-Rho), PLGA functionalized with antibodies (PLGA-Ab), PLGA functionalized with dendrons (PLGA-dendron), Ethyl cellulose NPs (EC) and cationic Ethyl cellulose NPs (EC cationic). NPs were measured individually by Atomic Force Microscopy (AFM) force spectroscopy and a multiparametric nanomechanical study was performed including the determination of the Young'ss modulus, breakthrough force, total indentation and adhesion, covering from small to large deformations and the NPs' rupture, thus containing all relevant mechanical information.Results showed an effect of composition, functionalization and loading on the NP mechanics evaluated and a graphical representation method has been proposed to identify formulations with similar properties.

POSTER 74 presented by:

NAME: James Eills
GROUP: Molecular Imaging for Precision Medicine
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Organ-on-a-Chip Research meets MRI: Exploring Type 1 Myotonic Dystrophy

Myotonic dystrophy type 1 (DM1) is a life-threatening and chronically-debilitating disease. Diagnosing DM1 and evaluating response to treatments is currently done via invasive methods; a rapid and non-invasive tool to study DM1 would be game-changing. Since DM1 alters cellular metabolism, changes in metabolic flux can be observed to track the disease progression. Magnetic resonance imaging (MRI) would be an ideal method for this purpose, since it is noninvasive and provides a unique signature for individual metabolites, but the sensitivity first needs to be improved.Parahydrogen-induced polarization (PHIP) is a technique that can be used to hyperpolarize molecules in solution, meaning the molecules exhibit MRI signals enhanced by a factor of around 100,000. In our lab we are developing PHIP as a method to hyperpolarize the metabolites fumarate and pyruvate; two molecules that are used as novel contrast agents for medical imaging. This involves chemically reacting a precursor molecule with para-hydrogen gas to yield a hyperpolarized product, and then purifying it from the reaction solution, ready for application. We are developing organ-on-a-chip (OOC) microfluidics as a platform to carry out these experiments on micro-scale human muscle tissue (healthy and DM1), and PHIP is an ideal hyperpolarization method to couple with OOC microfluidics. Both methods are famously low-cost and yield a high experiment turnover rate, making this an exciting new platform with which to study disease and treatment response.

POSTER 75 presented by:

NAME: Jiangqi Feng
GROUP: Nanoimmunology
INSTITUTION: University College London

A polymersomes that can act as an anti-inflammatory drug in mouse pulmonary fibrosis model

In our latest study, it was found that Poly (2-methacryloyloxyethyl phosphory-lcholine)-poly(2-(diisopropylamino) ethyl methacrylate) (PMPC-PDPA) polyme-rsomes can be used to target monocytes *in vivo* due to their ligand-protein interactions. Therefore, we wondered whether the polymersomes would be involved in regulating immune cells. So, we performed experiments on a bleomycin-induced pulmonary fibrosis (PF) mouse model of. Promisingly, after the 21 days intraperitoneal injection of PMPC-PDPA polymersomes, the fibrosis of PF mice was significantly recovered. We then assessed the changes in the lung immune microenvironment before and after the treatment in PF mice of different disease durations by flow cytometry based on whole lungs of mice. Using the same method, the uptake of DiD labelled polymersomes by different immune cell subsets was quantified as well. Through the immunofluorescence analysis of lung cryosections, we statistically quantity the immune cell subsets in the spatial distribution of lungs during the progression of pulmonary fibrosis. The relationships between different immune cell groups in the anatomy of the lung structure have also been revealed. After transparentizing the perfused lungs, we used light-sheet microscopy to image lung lobes to semi-quantify the immune cell uptaking of DiD labelled polymersomes from a 3D perspective. At the later stage, our research will focus on the anti-inflammatory/anti-fibrotic mechanisms of polymersomes. The essential pathways will be confirmed using techniques such as RNA sequencing. After that, further experiments will be carried out using mice knocked off the target gene.

POSTER 76 presented by:

NAME: Rosalina Gavín Marín
GROUP: Molecular and cellular neurobiotechnology
INSTITUTION: University of Barcelona

Early detection of miR519a-3p in Alzheimer'ss Disease patients and its relationship with cellular prion protein down-regulation in disease

MicroRNAs (miRNAs) are non-coding RNAs between 19 and 25 bp. They induce post-transcriptional gene silencing by binding to the 3'-UTR of complementary messenger RNAs and causing either degradation or inhibition of translation. Clinical relevance of miRNAs as biomarkers are growing because for its high stability and detection in circulating fluids. In this sense, diagnosis at asymptomatic stages of Alzheimer'ss 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. Many studies have determined that the expression pattern of some miRNAs is deregulated in AD patients, but to date, none has been correlated with down-regulated expression of PrPC during disease progression. That is why by means of cross studies of miRNAs up-regulated in AD with in silico identification of potential miRNAs-binding to 3'UTR of human PRNP gene, we selected miRNA519a-3p to our study. Other family members of miRNA-519 have been shown to bind to the 3'UTR region of PRNP *in vitro* and presumably degrade PrPC mRNA. In addition, up-regulation of some of them has been reported in different tissues from AD patients, including cerebrospinal fluid, plasma and serum. In fact, miR-519d-3p is pointed as a bridge regulator between mild cognitive impairment and severe AD. However, none of the studies address the prodromal stages of the disease or the expression profile of mi519 in other neurodegenerative diseases that also present dementia. Thus, in this study we analyzed miRNA519a-3p expression in samples from different brain parenchymal areas of AD at different stages of evolution as well as other neurodegenerative diseases such as PD. Our results show the specific and early up-regulation of miR-519a-3p since Braak I stage AD, suggesting its potential use as biomarker of preclinical stages of the disease.

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POSTER 77 presented by:

NAME: Subhadip Ghosh
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Shuttling Between the Chemotaxis of Symmetric and Asymmetric Enzyme-Powered Protocells

The self-propelled, synthetic active matter that transduces chemical energy into mechanical motion are examples of non-equilibrium systems with diverse applications in nanomachinery, fluidics, sensing, and transport. In this context, enzyme-powered propulsion is a classic model where the catalytic reaction has been utilized either by encapsulating enzymes within nanocarriers or tethering them to the vehicle chassis. Following observations on free swimming enzymes, in a recent study, we have shown phospholipid vesicles, when reconstituted with a transmembrane enzyme, were able to display motile behavior in a medium of their corresponding substrate solution. Using a combination of single-molecule fluorescence and optical microscopy analysis, these enzyme-powered liposomes exemplified substrate concentration-dependent propulsion. In addition, we observed chemotactic migration towards the substrate channel under a confocal microscope when these active liposomes were exposed to a substrate gradient inside a microfluidic channel. Interestingly, upon decorating the liposomes with surface-bound cues, they could adapt and reconfigure their chemotactic motion depending on surface-mediated interactions with solute molecules. We anticipate our results to constitute the first steps in fabricating multifunctional hybrid motors for carrying out specific functions under physiological conditions. We also shed light on cellular motility and chemotactic mechanism exhibited by microorganisms. We are focussing on how asymmetry within a protocell impacts its chemotactic propulsion. By rational encapsulation of active enzymes and incorporating membrane pores to facilitate the catalytic reaction in a gradient of the substrate, we are observing the modulation of the surrounding fluid environment and the subsequent chemotactic response of the protocell when there is a gradual transition in pore distribution from asymmetric to symmetric on their surface.

POSTER 78 presented by:

NAME: Barbara Ibarzo Yus
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

PMPC-PDPA Polymersomes in Cancer Vaccine development

Extensive efforts have been put towards developing efficient cancer vaccines. However, achieving tumour remission using vaccines remains a challenge [1]. Nanomedicine stands as an alternative for enhancing both targeting of immunogens into Antigen Presenting Cells (APCs) and antigen cross-presentation to CD8+ T cells [2]. This work has explored the potential of polymer-based nanoparticles (polymersomes, POs) to trigger immune responses against solid tumours by acting as vehicles for protein-based cancer vaccines. To this end, we used the amphiphilic polymer Poly(2-(methacryloyloxy)ethylphosphorylcholine)-co-poly(2 (diisopropylamino)ethylmethacrylate), in short, PMPC-PDPA. The PMPC block contains a phosphorylcholine (PC) head that targets Scavenger Receptors (SRs), especially SR-B1 [3,4], which is highly expressed in APCs, potentially enhancing antigen uptake within these cells. The hydrophobic PDPA block is pH-sensitivity and in environments at pH lower than 5.5, such as late endosomes, it can trigger the disassembly of POs [5]. We hypothesise that the pH sensitivity of the polymer, together with the targeting to APCs, can potentially enhance antigen release into the cytoplasm. This can result in antigen processing within the proteasome, translating in higher antigen cross-presentation within type I Major Histocompatibility Complexes (MHC-I), which is essential for CD8+ T cell activation and tumour rejection [6]. In this sense, we have exploited PMPC-PDPA POs to target APCs in a cancer vaccine approach.Results and discussion Immunisations with POs encapsulating OVA (P(OVA)) in mice harbouring the transgenic melanoma tumour B16-OVA have proven enhanced survival and slower tumour growth rates. Analyses of the immune infiltration within the tumour mass have revealed higher numbers of lymphoid cells and enhanced recruitment of OVA-specific CD8+ T responses in mice immunised with P(OVA) together with adjuvant, compared to those immunised with just free protein plus adjuvant. Besides, vaccination with P(OVA) formulations triggered the expression of checkpoint molecules in CD8 T cells, like PD-1 or TIM-3. Immunisations with P(OVA) and anti-PD-1 antibodies resulted in further survival extension in mice. The mechanisms driving the enhanced response observed are being analysed by exploring the subsets of immune cells that take up the particles upon immunisations, revealing preferential targeting for APCs in lymphoid organs, namely spleen and LNs. In conclusion, PMPC-PDPA POs as antigen carriers in combination with an adjuvant could be used as therapeutic vaccine approach for melanoma.

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POSTER 79 presented by:

NAME: Mohit Kumar
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Dye Labeling and STED Based Super Resolution Imaging of Peptide Nanostructures

Supramolecular materials have gained substantial interest for a number biological and non-biological applications. However, for optimum utilization of these dynamic materials, it is important to visualize their structures at the nanoscale, in solution. Previous approaches for imaging these structures have utilized imaging methods like STORM, which has provided important insights, but suffers from drawbacks of complex sample preparation and long acquisition times, thus limiting real-time imaging of dynamic processes. Furthermore, most reported imaging methods require covalent functionalization of dye to the self-assembling molecule, making it challenging and prone to unwanted effects. We demonstrate a simple, non-covalent fluorescent labeling design for STED (Stimulated Emission Depletion) based super-resolution imaging of self-assembling peptides. This is achieved by in situ, electrostatic binding of anionic sulfonates of Alexa-488 dye to the cationic sites of lysine (or arginine) residues exposed on the peptide nanostructure surface. A direct, multiscale visualization of static structures reveals hierarchical organization of supramolecular fibers with sub-60 nm resolution. In addition, the degradation of nanofibers upon enzymatic hydrolysis of peptide could be directly imaged in real time, and although resolution was compromised in this dynamic process, it provided mechanistic insights into the enzymatic degradation process. Noncovalent Alexa-488 labeling and subsequent imaging of a range of cationic self-assembling peptides and peptide-functionalized gold nanoparticles demonstrated the versatility of the methodology for the imaging of cationic supramolecular structures. Overall, our approach presents a general and simple method for the electrostatic fluorescent labeling of cationic peptide nanostructures for nanoscale imaging under physiological conditions and probe dynamic processes in real time and in situ.

POSTER 80 presented by:

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

Neurological disorders affect differently blood-brain barrier transcytosis of therapeutic nanocarriers

Brain delivery of therapeutics remains a challenge due to the blood-brain barrier (BBB). Drug nanocarriers (NCs) targeted to transcytosis receptors on brain endothelial cells can help transport drugs from the apical (blood) side to the basolateral (tissue) side of the BBB. Yet, how particular neuropathies alter BBB transcytosis is unknown. Since many prevalent neuropathies, such as Parkinson'ss or brain cancers, involve abnormal cellular storage of undegraded substances and trafficking dysfunctions, transcytosis may be altered in these diseases. To begin to study this paradigm, we focused on the lysosomal storage disorders as proof-of-concept models. They are caused by well-known genetic alterations, leading to well-defined aberrant cellular storage and altered vesicular trafficking. As NCs, we used model polystyrene (PS) and PLGA nanoparticles targeted to ICAM-1, an endothelial receptor that enables NC transcytosis across BBB cell models and *in vivo* in mice. First, brain endothelial models of different neuropathies were generated and validated, including acid sphingomyelinase (ASM) deficiency, which causes Niemann-Pick type A disease, and glucocerebrosidase (GBA) deficiency, associated with Gaucher disease and Parkinson'ss. Then, PS and PLGA NCs coated with anti-ICAM or control IgG were prepared and characterized. NCs had ≈200 nm diameter, 0.17 PDI, -30 mV _-potential, and 200 antibody molecules/NC and showed specific binding to ICAM-1 on brain endothelial cells (50-fold over IgG NCs). Confluent cell monolayers grown on transwells showed formation of cellular junctions, TEER raising over time, and <2% NC leakage, demonstrating barrier function. Flow cytometry showed that ASM and GBA deficiencies increased ICAM-1 expression. Binding of anti-ICAM NCs was increased in ASM deficiency but unaffected in GBA deficiency, while uptake was decreased in ASM deficiency and increased in GBA deficiency. These results can be explained by the fact that ASM-mediated generation of ceramide is necessary for CAM-mediated uptake and, thus, its inhibition decreases this. Instead, GBA deficiency leads to glucosyl-ceramide accumulation, which may mimic ceramide in enhancing CAM-mediated uptake. Contrarily and surprisingly, transcytosis was increased in ASM deficiency and decreased in GBA deficiency. This was because a fraction of transcytosed NCs was taken back into cells at the basolateral side and, just like uptake at the apical side, basolateral uptake was decreased in ASM deficiency and increased in GBA deficiency. Given that basolateral uptake was faster than apical uptake, GBA deficiency caused higher NC retention in BBB cells compared to ASM deficiency. These results highlight the intricacy of transcytosis, the fact that transcytosis can be followed by BBB reuptake, and that different diseases alter these processes differently, for which this type of study is key to guide the design of therapeutic drug NCs.

POSTER 81 presented by:

NAME: Catia Lopes
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Cargo avidity controls brain vasculature transcytosis and endocytosis: mechanisms and implication in Alzheimer disease

The blood-brain barrier (BBB) comprises polarized brain endothelial cells (BECs) phenotypically conditioned by the CNS. Transport across BECs is of paramount importance for nutrient uptake and ridding the brain of waste products. The intracellular sorting mechanisms that regulate successful receptor-mediated transcytosis in BECs remain is yet to be fully elucidated. We will present how BEC sort nutrient using cargo avidity to trigger endocytosis or fast transcytosis. Using a combination of experimental and computational biophysical tools and advanced imaging, we will show the role of BAR domain protein syndapin-2 on fast transport via tubule formation. We present evidence for the impact of the avidity of amyloid-_ assemblies in their trafficking across the brain endothelium and in low-density lipoprotein receptor-related protein 1 (LRP1) expression levels, which may affect the overall clearance of amyloid-_ across the BBB. All of these suggest that risk factors for Alzheimer'ss disease, amyloid-_ expression and ageing, are associated with a decline in the native expression of syndapin-2 within the brain endothelium. Our data reveal that the syndapin-2-mediated pathway, and its balance with the endosomal sorting, are important for amyloid-_ clearance proposing a measure to evaluate Alzheimer'ss disease and ageing, as well as a target for counteracting amyloid-_ build-up.

POSTER 82 presented by:

NAME: Gerardo Arturo López Muñoz
GROUP: Biosensors for bioengineering
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Direct, Label-free, and Multiplexed Biosensing by Scalable and Lithography-Free Plasmonic Metasurfaces for Point-of-Care and Bioengineering

Plasmonic metasurfaces have been widely widespread in the last years, motivated by the recent advances in the nanofabrication field and the increasing demand for high throughput biosensing platforms at the Point-of-Care. The recent advances in electronics, microfluidics, and signal processing have enabled the complete development of highly integrated prototypes for the Point-of-Care and different bioengineering applications. However, the progress observed from a fabrication point of view has been remarkable, led by the potential benefits metamaterials can offer in plasmonic sensing: sensor miniaturization, multiplexing opportunities, and extreme sensitivity biodetection. Although conventional top-down approaches, i.e., electron-beam lithography, have been extensively employed to develop plasmonic metasurfaces for biosensing, lithography-free bottom-up nanofabrication strategies based on nanopatterned thin-films by Glancing Angle Deposition (GLAD) and Thermal Dewetting (TDW) are candidates to surpass the limitations of top-down lithographic techniques with large-scale and high-throughput fabrication processes for 2D and 3D plasmonic metasurfaces over a broad material set. We focus on the challenges and opportunities to achieve lithography-free plasmonic metasurfaces by combining GLAD and TDW in single fabrication processes to conduct scalable and high-throughput plasmonic metamaterials for direct, sensitive, and multiplexed metaplasmonic biosensors for Point-of-Care and bioengineering applications.

POSTER 83 presented by:

NAME: Gerardo Arturo López Muñoz
GROUP: Biosensors for bioengineering
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Surface Plasmon Resonance Assay for Label-free Detection of Autoantibody-Mediated Complement Activation in Patients with Myasthenia Gravis

Myasthenia Gravis (MG) is a rare autoimmune disease mediated by antibodies that target the neuromuscular junction (NMJ). Approximately 80-90% of patients display antibodies directed against the acetylcholine receptor (AChR). The postsynaptic membrane is depleted of AChR causing a compromise of neuromuscular transmission. Detection of Anti-AChR antibodies confirms an MG diagnostic, these antibodies can act through three different pathogenic mechanisms: blockade of the AChR channel function, cross-linking of AChR by internalization in the muscle cells by endocytosis and destruction of the receptors, and complement activation leading to the destruction of the neuromuscular junction through the production of membrane attack complex (MAC). Clinical assays measure only antibody binding but cannot differentiate the main mechanism in each patient. The design of a high-throughput and scalable plasmonic biointerface is highly attractive for Point-of-Care (POC) and portable devices. This study aims to develop cellular 2D and 3D models connected to a fluidic system with a Label-free plasmonic biosensor that measures AChR autoantibody-mediated complement activation that will help with a more accurate diagnosis of MG. The immobilization of cys-Protein G on the SPR gold detecting surface for an oriented antibody binding was optimized. In addition, the physioabsortion of anti-AChR to the gold surface was also characterized in terms of selectivity and sensitivity highlighting a better analyte-antibody binding affinity for the assay.

POSTER 84 presented by:

NAME: Elisa Mastrantuono
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Exploiting ligand multivalency to selectively target HER2 receptor in Glioblastoma

Glioblastoma (GBM) is the most common and fatal primary malignant tumour in the central nervous system, and the available therapies are still limited. The tumour microenvironment and the blood-brain barrier pose significant obstacles to the delivery of drugs into the brain, making selective targeting not trivial to achieve. Polymeric micelles functionalised with selective ligands are now emerging as powerful vectors to reach into one of the most difficult organs in the human body.In this study, we focus on the role of the human epidermal growth factor receptor 2 (HER2). Its dimerization with other ligand-bound HER family receptors causes the uncontrolled growth of cancer cells. For this reason, HER2 overexpression in brain cancer can represent a poor prognostic marker in patients with GBM and, therefore, is becoming a promising therapeutic target. This work aims to identify the optimal number of ligands (KCCYSL peptide) against the HER2 receptor and tethered on spherical polymeric micelles. Here we analyse how the ligand multivalency influences the interaction between the micelles and the GBM cells and the ability of the ligand to bind HER2, inhibiting its activity and thus inducing cytotoxicity and cell death.

POSTER 85 presented by:

NAME: Diana Matias
GROUP: Molecular bionics
INSTITUTION: University College London

Polymersomes with tunable avidity to LRP1 in health and glioma model

The healthy blood-brain barrier (BBB) guarantees homeostasis by restraining most molecules and pathogens from entering the brain. In cancer, the tumour cells highjack BBB creating a heterogeneous blood vessel network, augmenting their growth, known as a blood-tumour barrier (BTB)1. We hypothesised that glioma cells modulate the expression of several molecules involved in the mechanisms across the brain endothelial cells (BECs), including receptor-mediated transcytosis (RMT)2,3. However, the precise molecular mechanisms underlying such changes in the BECs remain elucidated. Here, we uncover the alterations in the trafficking across BECs in healthy and tumour vasculature *in vitro* and *in vivo* by exploring how the affinity binding could influence the BTB-shuttles by using LRP1- targeted polymeric nanoparticles (polymersomes) functionalised with specific angiopep2 moieties in murine glioma model compared to healthy mice.We show that the functionalisation with high angiopep2 moieties on the surface of pH-sensitive poly (ethylene glycol)-block-poly(2 poly(2-(diisopropyl amino) ethyl methacrylate) (PEG5k-PDPA100) polymersomes is crucial for BTB crossing compared to healthy vasculature. We confirm that crucial proteins' expression for RMT, including protein kinase C and casein kinase II interacting proteins family (PACSIN), dynamin-3 and -2, clathrin light chain B and low-density lipoprotein receptor-related protein 1 (LRP1), are altered in the BTB compared to BBB, *in vitro* and ex vivo. The downregulation of LRP1 levels on BTB may be due to its shedding by detecting increased soluble LRP1 levels in the bloodstream compared to healthy mice. Furthermore, RNA sequencing analysis of brain vasculature from proneural PDGFR+/TP53- murine glioma model show that trafficking signature of the vasculature of tumour bulk is different from healthy. We conclude that BBB and BTB are phenotypically different. Moreover, molecules upregulated in BTB could be good candidates to target the tumour vasculature, such as GLUT-1, syndecan-IV and CD81.

POSTER 86 presented by:

NAME: Víctor Mejías Pérez
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

MR1 as a sensor for intracellular infection

Tuberculosis (TB) remains a major challenge to global health. According to WHO, around 1/4 of the world'ss population is infected by Mycobacterium Tuberculosis (Mtb), the bacillus responsible for the disease, causing 1.5 million of deaths in 2021. The lack of new drugs and the development of drug resistance means that patients are subjected to daily administration of cocktails of anti-TB drugs during months or even years, causing strong hepatic and neurological toxicity. Nanoparticles are an excellent vehicle to deliver drugs, increasing their half-life in the body, and inducing a clinical response with reduced dosage and fewer side effects. Human T cells have evolved to recognise a variety of protein and non-protein antigens produced by mycobacteria. In particular, a subset of unconventional T-cells has shown to be restricted by MHC class I-like related (MR1) proteins. MR1 is a highly conserved and low-polymorphic protein that at steady-state is localised in the Endoplasmic Reticulum, but which is promptly translocated to the cell surface upon binding of mycobacterial metabolites and association with _2-microglobulin (_2m), effectively functioning as a sensor for intracellular infection. We aim to create a peptide library using phage display that binds specific MR1-metabolite complexes to directly target antibiotic loaded polymersomes to infected host cells. We have successfully expressed and purified the MR1/_2m complex in E. Coli BL21, which will be used as a target for the following Phage Display experiments.

POSTER 87 presented by:

NAME: Ruben Millan-Soslsona
GROUP: Nanoscale bioelectrical characterization
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Mapping the Membrane Capacitance in Living neurites at the Nanoscale by In-liquid Scanning Dielectric Microscopy

Traditional disease detection techniques require a costly and complex labeling process and extensive biochemical assay. However, through various experimental results, it has been established that nonbiological properties, such as mechanical, electrical, and optical parameters of cells, also change during disease and undergo pathological changes. .1-3 Sometimes, nonbiological parameters can show an early sign of disease before significant changes occur. Therefore, the study of the specific capacity of the membrane can play a relevant role in biomedicine. The specific capacitance of a neuron's membrane influences synaptic efficiency and determines the speed of propagation of electrical signals along dendrites and axons. The value of this important parameter remains controversial because of the difficulty of its measurement and the wide range of the reported value4. In addition, many of the techniques used provide information at the whole cell or culture level and in many cases are invasive techniques. Therefore, we propose here to use the latest advances in in-liquid scanning dielectric microscopy (in-liquid SDM)5,6 to measure this important property with nanoscale resolution in neurites from primary cultured cortical neurons under live conditions. In conducting the experiments, special attention was paid to maintain physiological conditions throughout the experiment. We show the first electrical images made on neurites under live conditions. For this purpose, the EFVM 7 technique has been used, which, in addition to acquiring electrical data, allows us to obtain mechanical properties. These results open the door to the realization of capacitance maps and mechanical maps at the nanoscale for the study of cells.

POSTER 88 presented by:

NAME: José Muñoz-López
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Design and characterization of bifunctional Janus micelles

Janus micelles consist of a hydrophobic core and a corona composed of two well-defined hydrophilic hemispheres. Such a "two-faces", like the roman god Janus, arrangement form from the self-assembly of ABC triblock copolymers, in which A and C are hydrophilic polymers that dislike each other, forming the two faces, and B hydrophobic block forming the core. In this work, we first studied the controlled polymerization of N-vinyl-2-pyrrolidone (NVP) by atom transfer radical polymerization (ATRP) to form poly(N-vinylpyrrolindone) (PVP). When the conditions for controlled polymerization of NVP were optimized, the synthesis of the triblock copolymer poly(ethylene glycol)-polylactide-poly(N-vinylpyrrolidone) (PEG-PLA-PVP) was studied and carried out in two main steps. First, PEG-PLA diblock copolymer was synthesized by the ring-opening polymerization (ROP) of PLA with commercial PEG. Second, the triblock copolymer was synthesized by ATRP of NVP with the PEG-PLA-Br macroinitiator derivate from the latter synthetic step. Polymer and nanostructures analysis and characterizations have been done by Gel Permeation Chromatography (GPC), Nuclear Magnetic Resonance (NMR) and Dynamic Light Scattering (DLS). Finally, the functionalisation of both coronas with different targeting ligands is in the pipeline.

POSTER 89 presented by:

NAME: Rui Pereira
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Targeting medulloblastoma in a unique in utero tumor model on immunocompetente mice

In the present work, using a specific immune-privileged developmental time window, we demonstrate the possibility to generate a novel *in vivo* human medulloblastoma (MB) model in an immunocompetent (IC) animal. Human MB mcherry/NanoGlo positive cells, after in utero injection in the telencephalic ventricle of IC embryos at E14.5, engrafted, integrated and formed tumor masses in the embryonic mouse brain. MB cells persisted their growth and invasion process in postnatal brains recapitulating tumor associated features such as infiltration, de novo angiogenesis, presence of reactive astrocytes and activated microglia. We believe that our new in utero model will better elucidate intrinsic mechanisms of MB tumor engraftment, invasion, growth and blood brain barrier (BBB) development and interaction in IC host that will lead to the use of this to corroborate nanodelivery strategies of therapeutic agents across BBB.

POSTER 90 presented by:

NAME: Peter Pfeifer
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Design and synthesis of novel block-copolymers for applications in precision medicine

Modern therapeutical approaches such as Targeted Drug Delivery systems or Precision Medicine have emerged from interdisciplinary research. The use of polymeric particles as carriers in drug delivery has drawn great attention due to their versatility, biocompatibility and the possibility to tailor new materials to almost any desired characteristics. Different amphiphilic diblock and triblock-copolymer architectures are perfect candidates for forming nanometric vesicles and micelles. This structure permits these units to hold any cargo, which makes them the ideal carrier in drug delivery systems. Through conjugation of biologically active molecules such as peptides, proteins, antibodies, sugars, etc., onto the surface of these polymeric vesicles, cell recognition, attachment, and trans cytosis can be greatly enhanced. The present project aims to synthesise and characterise of new polymeric materials able to self-assemble into defined nanoscopic units. We are synthesising diblock and triblock copolymers with AB, ABA and ABC structures. These materials will be designed, so that the cargo molecule of therapeutic interest in the traditional drug delivery systems will either form part of the polymeric chain or be attached to it, thus eliminating the need for subsequent drug encapsulation steps. Sophisticated controlled or living polymerisation methods, such as RAFT and ATRP will be used for polymer synthesis. Polymersome formation through self-assembly will be studied through film rehydration, solvent displacement, solvent switch and pH switch. The polymer vesicles need to be labelled or tagged with a reporter unit for detectability and traceability and conjugated with a biologically active molecule for cell recognition and uptake to obtain a functional drug delivery system. Two strategies will be investigated linking fluorophores and peptides to the polymeric chain. We will optimise coupling reactions before and after self-assembly to open up more possibilities for polymer functionalisation. Besides basic Click-chemistry techniques, other bioconjugation methods will also be applied. he final plan of the investigation is to elaborate a library of protocols for each step of the formulation process. These protocols would cover the design principles for new polymers bearing therapeutic agents, the synthesis of principal copolymer architectures from monomers of distinct chemical functionalities, the self-assembly procedure to obtain different nanoparticle sizes and morphology and finally, the series of conjugation reactions for the coupling of the reporter and biologically active molecules to the nanometric vesicles.

POSTER 91 presented by:

NAME: Giulia Maria Porro
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Labelling the blood-brain barrier with artificial targets by identifying selectively retained peptides on brain endothelial cells

The Blood-Brain Barrier (BBB) is an impermeable barrier composed primarily of specialised brain endothelial cells (BECs) that represents a major obstacle to treating neurological disorders by preventing the delivery of therapeutics into the brain. Current strategies to transport drugs across the BBB target proteins associated with, but not exclusively expressed on BECs; hence, such strategies result in off-target drug delivery to peripheral organs, thereby limiting brain specificity. We aim to generate artificial targets by identifying peptides selectively retained on the surface of BECs to avoid off-target drug delivery. This is achieved by exploiting the BECs lower internalisation rate compared to other ECs. Using phage display technology on primary brain, liver, and lung ECs we selected, based on time, a binding peptide population and a retained peptide population for each organ. Notably, the results showed that for all the cell types the binding population displays heterogeneity without a unique brain-specific peptide while the retained population is highly homogenous; BECs show at least three retained brain-specific peptides. Currently, we are quantifying the different internalisation rates between BECs and peripheral ECS for the retained brain-specific peptides identified.

POSTER 92 presented by:

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

Detection of protein aggregation in Leishmania infantum parasites

The leishmaniases are a group of poverty-related parasitic diseases transmitted by sandflies and caused by protozoa from more than 20 Leishmania species (WHO, 2021). Leishmania infantum has been chosen for this work, as it is the main species causing human leishmaniasis in the Mediterranean region (World Health Organization, 2019). Current medicines for the treatment of human leishmaniases are expensive, they have high systemic toxicity and limited efficacy, and drug resistance is becoming increasingly common (Ghorbani & Farhoudi, 2018; Ponte-Sucre et al., 2017). Thus, developing new therapeutic strategies against Leishmania and identifying new molecular targets are urgent needs.L. infantum promastigotes were strongly stained with a dye that detects the presence of intracellular protein aggregates in live cells. Through a proteomics approach we identified 145 aggregative proteins in parasite cell extracts. After an in silico analysis of this protein pool, three short peptides with amyloid fibre-forming capacities were selected and their aggregation propensity was characterized by thioflavin T fluorescence measurements. When these endogenous peptides were administered to L. infantum *in vitro* cultures, with the aim of stimulating protein aggregation in the parasite, its viability was not significantly affected. On the other hand, amyloid pan-inhibitors from a family of _-sheet intercalators (Espargaró et al., 2019), clearly reduced promastigote viability *in vitro*. The results presented suggest that inhibiting protein aggregation in the parasite could represent an innovative strategy to address the problem of the lack of efficient antileishmanial treatments.

Acknowledgements:
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POSTER 93 presented by:

NAME: Lorena Ruiz Perez
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Structural Biology in the Liquid State: shedding light on Protein Dynamics

Liquid phase electron microscopy LP EM offers remarkable capabilities with regard to imaging time-resolved structures in their native liquid media by removing the artifacts caused by traditional drying or cryogenic treatments. One of the most exciting applications of LP EM is the investigation of cell molecular machinery structures such as proteins. The liquid nature of the sample offers novel opportunities such as accessing previously inaccessible protein states or the possibility of 3D structure reconstruction by applying tomographic methods. Indeed, the free movement of soft objects in LP EM grants the opportunity of screening the protein structural landscape during the imaging process. We propose the combination of all-atom simulations with LP EM to complement protein structural studies with dynamic investigations. We employed LP EM to image proteins in solution exploiting their natural rotation with the aim of accessing the particle structural landscape. Tomographic techniques were employed for reconstructing the 3D structure of Apoferritin. The use of LTEM for the investigation of proteins is not limited to 3D reconstruction and structural analysis. We have also used LP EM to investigate Amyloid-_ (A_) aggregation. A_ is a small, disordered protein. A_ accumulates into stages of microscopic amyloid oligomers, fibers, and plaques that are found in brains affected by Alzheimer'ss disease (AD). The details of the aggregation pathway remain elusive, with much of current knowledge arising from computational simulations. Preliminary investigations on A_ aggregation, via LP EM will be presented. This work, although still in its early stages, promises to provide relevant and novel biological information on A_ aggregation pathways.

POSTER 94 presented by:

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

Hyaluronidase nanomotors reduce the viscosity of synovial fluid and enhance the diffusion of urease nanomotors

In the last decades, the development of nanoparticles that can increase the efficiency of clinical treatments has been studied. The use of passive nanoparticles has been reported to have low efficacy due to the need for overcoming the biological barriers present in the human body. One way of tackling this problem is by developing the so-called "active" nanoparticles, which interact with the media and change its rheology, reducing its viscosity. These viscous media are a challenge for the field of nanomedicine as passive particles are retained and trapped in the complex network, reducing their ability to move and reach the target site. However, the development of enzyme-powered nanomotors that can interact with these biological barriers and allow them to move within these fluids has not been well-studied yet. Actually, the study of the motion of nanomotors has been mainly performed in aqueous media. Nevertheless, most of the fluids present in our body are viscoelastic media such as synovial fluid present in the joints, mainly composed of hyaluronic acid. Here, we show that the combination of enzyme-powered nanomotors based on hyaluronidase and urease enzymes in different troops can interact with the complex media, reducing the viscosity of both simulated synovial fluid and ex vivo synovial fluid from sheep. Our results show enhanced diffusion of urease nanomotors when synovial fluid was previously treated with hyaluronidase nanomotors. These results pave the way for the use of nanomotors for joint injury treatment, improving therapeutic effectiveness, and achieving faster and more efficient delivery of therapeutic agents than traditional methods.

POSTER 95 presented by:

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

Catalase-powered nanomotors disrupt and cross an *in vitro* mucus model

Cancer chemotherapies used in the clinic have poor bioavailability and severe off-target effects, mainly due to their systemic administration. Drugs can be locally administered to a specific organ or area of the body to increase their concentration in the target and reduce side effects, although this approach is hindered by biological barriers such as mucus. Goblet cells, found in the intestine or the lung, secrete mucus to create a protective layer that prevents microorganisms, allergens, and other particles to reach the cells and damage them. However, this natural protection system acts as an obstacle to delivering localized therapy. Here we show how catalase-powered nanomotors can disrupt and cross mucus secreted from cells, in the presence of hydrogen peroxide, thanks to the oxygen bubbles produced from the reaction catalyzed by the enzyme. This strategy can overcome current treatment limitations by enhancing the number of particles that reach their target, thus holding potential for acting as drug delivery systems.

POSTER 96 presented by:

NAME: Meritxell Serra-Casablancas
GROUP: Smart nano-bio-devices
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Urease-powered nanobots show enhanced tumor accumulation in an orthotopic murine model of bladder cancer

Bladder cancer is one of the most common cancers worldwide. Conventional treatments, based on intravesical administration of drugs, show good survival rates but have low therapeutic efficacy. This may be due to the degradation of the drug by the harsh environment of the bladder or to the low accumulation/penetration into the tumor. Urease-powered nanobots could potentially overcome these limitations since they hold greater fluid mixing and exploration capabilities in comparison to current drugs or passive nanoparticles. Here, we describe the enhanced accumulation of urease-powered nanobots in an orthotopic murine model of bladder cancer. Nanobots, based on mesoporous silica nanoparticles, were functionalized with urease, radiolabeled and used for *in vivo* studies. With a novel label-free imaging method, based on Scattered Lightsheet Microscopy, and with Positron Emission Tomography (PET) imaging, taken after treatment, we show an enhanced accumulation (5-fold increase) in the tumor compared to their passive counterparts. These results add value to the previously reported properties of the nanobots and position them as promising drug delivery systems for bladder cancer therapy.

POSTER 97 presented by:

NAME: Shubham Tanwar
GROUP: Nanoscale bioelectrical characterization
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Exploring the use of in-liquid SDM to probe defects in Operating Electrolyte-Gated Organic Field-Effect Transistors

Electrolyte-Gated Organic Field-Effect Transistors (EGOFETs) are the focus of active research for a wide range of biosensing and bioelectronic applications owing to their low voltage operation, inherent biocompatibility, and the presence of highly sensitive interfaces [1]. Biorecognition events and bioelectronic processes modify and modulate the interfacial properties that are readily detected by the EGOFET devices. However, the defects at these interfaces can drastically affect signal transduction and integrity. As defects are ubiquitous in electronic devices, their detection and proper characterization are necessary to design approaches to minimize and mitigate their influence on the device performance. Probing the electrical properties of the semiconductor/electrolyte interface in a functional device has been a technological and scientific challenge, which recently has been overcome by implementing in-Liquid Scanning Dielectric Microscopy (in-Liquid SDM) on EGOFETs [2].Here, we have extensively employed in-Liquid SDM by automating the measurements coupled with further technical improvements to probe the semiconductor/electrolyte interface of an operating EGOFET. After understanding the operating mechanism of EGOFETs with in-Liquid SDM, we are exploring the nature of defects and their influence on the local electrical properties by comparing the regions with and without defects. We hope such exploration studies will provide valuable information to guide design rules for better and more efficient devices. The ability to visualize the influence of defects on the local electrical properties in a working device is a critical step in the eventual deployment of futuristic biosensing and bioelectronic devices in the real out-of-lab environment.

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POSTER 98 presented by:

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

Giving New Features to an Old Antibiotic: Cyclodextrin-Arginine-Ciprofloxacin "Hard" Ternary Complex Assembly

Designing novel functionalities in clinically validated, old antibiotics holds promise to provide the most economical solution for the global lack of effective antibiotics, as undoubtedly a serious health threat. Using the surface chemistry of the cyclodextrin (_CD) cycle and arginine (arg) as a linker, we developed a novel type of "hard" tertiary antibiotic complex (_CD-arg-cpx). In contrast to classical "soft" inclusion complexes, which only modify antibiotic solubility, the "hard" complex is significantly more stable and does not release the free drug. The complex intensifies interactions with bacterial membranes and increases the drug's availability inside bacterial cells, thereby improving its antimicrobial efficacy and safety profile. Novel generations of multifunctional antibiotics, formulated as drug delivery systems per se, that take the drug to the site of action, maximize its efficacy, and provide optical detectability are envisaged as the future in fighting against infections and as a prospective new tool against multiresistant strains.

POSTER 99 presented by:

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

A multiscale computational analysis of LRP1 collective binding capacity

The brain is the most energy-expensive organ in humans consuming around 20% of body's metabolic resting rate. At the same time, the molecular balance that leads to biochemical reactions in the brain makes this organ extremely delicate to alterations from the outside environment, that is, the blood circulation. Evolution made our bodies develop a special wall between neurons and the blood flux, the Blood-Brain Barrier BBB. Composed of endothelial cells that tightly wrap the capillaries, the BBB apply strict control over the molecules that enter and exit the brain. In this control, the membrane proteins called receptors play a fundamental role, binding to the molecules and activating the inward/outward transport mechanism. This research aims to understand how a particular receptor, the low-density lipoprotein receptor-related protein 1 (LRP1), interacts with the molecules and between themselves and how these interactions lead to transport. The study approaches the problem from a computational biophysical point of view, using the combination of atomistic molecular dynamics and molecular docking of the LRP1 receptor. These techniques' dynamics and binding information are then joined in a coarse-grained model that will extend the study to n>1 LRP1 receptors and then observe collective transport mechanisms.

POSTER 100 presented by:

NAME: Akhil Venugopal
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Dynamic Lipid Vesicles: Towards Adaptive Drug Delivery Systems

Living systems uniquely form active self-assembled structures, which consume chemical energy for their adaptive, and autonomous structural & functional responses.1 These dynamic structures can form and break by maintaining out-of-equilibrium processes through chemical transformations that drive dissipative adaptation and self-assembly.2 Inspired by the natural systems, there has been a surge in the development of out-of-equilibrium self-assembled materials.3,4 Herein, we present the formation of active self-assembled vesicles which function under out-of-equilibrium conditions resulting in the formation of vesicles with a programmable lifetime. As a proof of concept, we have synthesized a hydrophilic phospholipid synthon with terminal amine that convert to imines in the presence of long carbon chain aldehydes with labile ester group. Such imines become amphiphilic and thus self-assemble into vesicles. Conversely, the ester hydrolysis reverses the process driving vesicle disassembly. We, therefore, designed vesicles that exist in the presence of a constant fuel supply and can quickly adapt their shape and configuration as a function of environmental stimuli. We aim to prove the use of these transient lipid vesicles as an adaptive interface for targeted drug delivery.

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POSTER 101 presented by:

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

Engineering new cellular models to study ICAM-1 mediated transport of therapeutic nanoparticles

Intercellular adhesion molecule 1 (ICAM-1) is a transmembrane glycoprotein that represents a promising target for drug delivery because it is expressed on cells at sites of pathology. ICAM-1 targeted nanoparticles (NPs) specifically interact and enter human cells via cell adhesion molecule-(CAM)-mediated endocytosis. To advance this strategy toward translation, we developed new cellular models expressing ICAM-1 from clinically relevant animal models. ICAM-1 sequences from mouse, pig and monkey, along with human as a control, were cloned in mammalian expression vectors and transfected in HEK239T cells. Transgene expression on the cell-surface was verified by flow cytometry and confocal microscopy. These cellular models were established chemically, then used to test cell interactions of model polymeric anti-ICAM-1 NPs, which had 209.1 + 7.2 nm, 0.17 + 0.03 polydispersity index, -14 mV _-potential. Data showed specific cell targeting compared to both control IgG NPs and cells treated with anti-ICAM-1 as a blocker. Anti-ICAM NPs entered cells at 37ºC but not 4ºC, in a time dependent manner, and inhibitors showed this occurred via amiloride-sensitive CAM-mediated endocytosis, with NPs further trafficking to lysosomes. Altogether, these data indicate this new cellular platform behaves similarly to human cells naturally expressing ICAM-1, representing a valuable tool to study and test ICAM-1 targeted NPs in cellular models complementary to animal models used for translation.

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POSTER 102 presented by:

NAME: Zhendong Xie
GROUP: Molecular bionics
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

The physiologically based pharmacokinetic (PBPK) modelling of superselective nanomedicine

The poly(2-methacryloyloxyethyl phosphorylcholine)-poly(2-(diisopropylamino)ethyl methacrylate) (PMPC-PDPA) polymersomes are proved to be super selective to the cells toward antigen-presenting cells. We can thus make precision nanomedicine treating diseases with very low dose injection and neglectable side effects. We employed physiologically based pharmacokinetic (PBPK) modelling to rebuild and preview the pharmacokinetics of the polymersomes combined with the diffusion data *in vivo*. The PBPK model contains the circulatory system and organs being treated. The net-like PBPK model obeys the connection rules among these compartments, and the absorption, distribution, metabolism, and elimination (ADME) process is simulated by functions such as the first order absorption.We input data of the dose injected, and concentration-time profile of each compartment in the model will be given. Then, we use the super selectivity theory to calculate the association constant KA/j between the polymersomes and different organs. The polymersomes are super selective to the organs with large KA/j. By modifying the parameter in the model relying on the calculated KA/j, we can get a more accurate model. We can make more efficient administration strategies and predict the results based on the PBPK model.

POSTER 103 presented by:

NAME: Celia Ximenes-Carballo
GROUP: Biomaterials for regenerative therapies
INSTITUTION: Institute for Bioengineering of Catalonia (IBEC)

Targeting wound healing with CaZn releasing platforms

Chronic wounds are a major socioeconomic burden, with high costs and pain associated with extensive and inefficient treatments. Appropriate diagnosis and treatment will diminish costs and increase the patient'ss life quality. So, research has focused on the development of new wound healing devices. However, a device enabling fast-effective closure at a low cost/effectiveness ratio is still missing.Ions such as calcium (Ca2+) and zinc (Zn2+) are essential for skin homeostasis. Ca2+ is involved in platelet aggregation, epidermal stratification, and collagen synthesis. On the other hand, Zn2+ deficiencies had been long associated with impaired wound healing. This work aims to develop ion-releasing platforms based on CaZn for wound healing.Submicrometric particles incorporating Ca2+ and Zn2+ were synthesized, with sustained release at the physiological levels. An exhaustive characterization of their composition and reproducibility was performed. *In vitro* results revealed that these particles stimulate collagen and VEGF production, cell migration, and decreased collagen contractibility. These particles may have the potential to be used for hard-to-heal wound healing therapies.

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