Bioengineering for Future & Precision Medicine

13th IBEC Symposium

IBEC

Institute for Bioengineering of Catalonia

EXCELENCIA SEVERO OCHOA

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Welcome to IBEC's 13th annual symposium

The world has changed dramatically since our last symposium due to the COVID-19 outbreak. Despite the difficulties that the pandemia has posed on our work and daily lives, I am proud that, thanks to the effort of all IBEC community, we have been able to adapt and continue with our activities.

I am also happy to meet you again in our annual symposium, to learn from our keynote lecturers, to have an overview of IBEC scientific activities through our posters and flash presentations and to discuss how we can continue working with research centres, hospitals and industry to accelerate the uptake of bioengineering based solutions for health into the clinics. The IBEC annual symposium brings together high-profile international experts for an open forum for interdisciplinary discussions and networking.

This year the 13th IBEC Symposium will focus on one of our three main areas of application of research at IBEC: Future and Precision Medicine.

We're delighted that you've joined us for our symposium, and hope that you'll be stimulated and inspired by our programme of talks, posters, and networking.

Josep Samitier
Director

Bioengineering for Future & Precision Medicine

13th IBEC Symposium

Programme

Tuesday 27th October · 13th IBEC Symposium for Future & Precision Medicine

09:15 - 09:35	Opening ceremony
09:35 - 10:00	Keynote speaker IBEC's Evolution and Scientific Highlights. **Josep Samitier** · *Institute for Bioengineering of Catalonia (IBEC)*
10:00 - 11:00	Round Table · Chair: Nuria Montserrat Clinical translation of 3D in vitro and organoids systems to model diseases and accelerate drug development. **Raquel Yotti**, **Maurice Whelan** and **Anton Ussi**
11:00 - 12:00	Flash Presentations
12:00 - 16:00	Break
16:00	Poster session

Wednesday 28th October · 13th IBEC Symposium for Future & Precision Medicine

15:00 - 15:25	Keynote speaker · Chair: Teresa Sanchis Biomimetic materials in medicine and beyond. **Javier G. Fernández** · *Singapore University of Technology and Design, Singapore*
15:25 - 16:00	Keynote speaker · Chair: Pere Roca-Cusachs Surface control of adhesion and growth factor mechanical and chemical crosstalk. **Ada Cavalcanti-Adam** · *University of Heidelberg, Germany*
16:00 - 16:40	Flash Presentations
16:40 - 17:40	Keynote speaker · Chair: Josep Samitier Microtechnologies and nanotechnologies in Drug Delivery. **Robert Langer** · *Massachusetts Institute of Technology (MIT), USA*
17:40 - 17:50	Time for PhD Committee
17:50 - 18:00	Time for PostDoc Committee
18:00 - 18:15	Awards and closing ceremony

Keynote Lectures

IBEC's Evolution and Scientific Highlights

Josep Samitier

Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain
University of Barcelona (UB), Spain

Over the past months, the important role of research and innovation and multidisciplinary and multisectoral collaboration has been stressed more than ever. In the fight against COVID-19, bioengineering has proven to play a very important role. From diagnosis with the use of nanosensors or e-health tools to treatment with targeted therapies, through the design of vaccines using nanomaterials or the identification of new drugs using in vitro models of organoids.

IBEC researchers have been involved in different initiatives against COVD-19, like the publications of Prof. Nuria Montserrat about the use of organoids to test the SARS-CoV-2 infection mechanisms and their participation in the IMI H2020 funded international consortia "Modern approaches for developing antivirals against SARS-CoV 2 interactions", the phenotypic targeting of infected cell for viral eradication by Prof. Guisseppe Battaglia, the development of e-tools for the early identification of patients at home using acoustic cough analysis (Prof. Raimon Jané), the use of functionalized nanoparticles for the detection, blocking and elimination of the virus (associated researcher Prof. Carlos Aleman from UPC), the virtual screening of drugs by computer protein-ligand docking analysis (Prof. Pere Roca-Cusacs) or the study of the impact of confinement measures on mental health and well-being (Prof. Paul Verschure)

We are also very proud of the participation of IBEC in the ORFEU programme, the mass screening platform against COVID-19 jointly set up by CRG-CNAG, IRB Barcelona and IBEC that analysed more than 35,000 samples using the PCR technique thanks to the work of more than 30 volunteers from IBEC.

It is necessary more than ever to continue advancing and generating knowledge in bioengineering, in order to face the new health challenges that may appear in the next few years in the most efficient way possible. In this sense, we can be very satisfied with our scientific outputs. By 2019, IBEC researchers have achieved our own record of 174 indexed scientific papers, 81% of them in the first quartile. Despite the partial lockdown of IBEC, in 2020 we have already 150+ indexed papers, so we are in a position to beat our own record!.

Moreover, in 2019 our younger researchers presented 10 new PhD theses, one of them double appointed with the TU Eindhoven.

Regarding competitive funding, our researchers Xavier Trepat, Elena Martínez, Samuel Sánchez and Loris Rizzello have achieved this year the highest distinctions from the European Research Council, obtaining 4 new ERC grants. Moreover, Elena Martínez, Xavier Trepat and Pere Roca-Cusachs have received funding from the third edition of the "Health Research Call" from the "La Caixa" Foundation for 2 projects aimed to used bioengineering against cancer.

Since last year, our researchers have obtained several recognitions. Prof. Javier Ramón has been distinguished as ICREA professor. Prof Nuria Montserrat won the XXXI Íñigo Álvarez de Toledo Award for Basic Research, while Dr. Pere Roca-Cusachs was chosen to join the European Molecular Biology Organization (EMBO). Prof. Samuel Sánchez was elected new member of the Young Academy of Spain and Rossella Castagna, a postdoctoral researcher in the Nanoprobes and Nanoswitches group at IBEC, was awarded with the ISOP2019 prize at the 9th International Symposium on Photochromism.

Regarding internationalization, we have signed an agreement with the Institute for Complex Molecular Systems (ICMS), a research institute of the Eindhoven University of Technology to share resources, knowledge and provide mobility programs, consolidating a collaboration that started in 2018. We have also signed a 5-years agreement with The European Molecular Biology Laboratory (EMBL) to set up a working framework for activities between EMBL and IBEC which support strategic long-term scientific and general collaboration in areas of mutual interest. In this framework, the first EMBL-IBEC Winter Conference on Engineering Multicellular Systems was organized last February with a huge success. The opening ceremony was led by Mrs. Ada Colau, the mayor of Barcelona, who also visited IBEC in May, interested in our research against COVD-19.

But IBEC has also performed at the highest level in clinical collaborations and technology transfer. In 2019, we have contributed to clinical highlights such as a pioneer operation designed to repair the heart tissue of a patient with a bioengineered implant, the development of mHealth technology to detect sleep apnea at home or the use of virtual reality to treat speech disorders. We have submitted 2 new patents, achieving a total of 8 patents active under valorization programs. We have also signed 7 new contracts with Catalan and international companies. More recently, Rob Surgical, the spin-off created by IBEC and the UPC, closed a €5 million investment

round with Scranton Enterprises. As a result of all these efforts, IBEC received last November the FEI Prize as an “Innovation supporting Institution of the year”

Regarding the social impact, we are proud to have reached in the last year millions of people with our press and communication activities, more than 3000 students with our outreach activities and to have been able to initiate a new collaboration with an innovative educational program, the “Magnet alliance” which aims to fight against school segregation. We have also renewed our accreditation as a unit of scientific culture and innovation.

I would not like to end this summary of IBEC activities since last symposium without doing a tribute to the memory for Prof. Fausto Sanz, founding Group Leader of IBEC, Professor at the University of Barcelona and mentor of many scientists of IBEC, who passed away last April.

I look forward to this 2020 symposium devoted to Bioengineering for future and precision medicine. Looking to the future and to bring optimism to the difficult times in which we find ourselves, we want to show how bioengineering can provide innovative solutions to guarantee equitable access to health and a better quality of life for all the citizens.

Josep Samitier *is Director of the Institute for Bioengineering of Catalonia (IBEC), group leader of the Nanobioengineering Group at IBEC and Full Professor of Electronics and Biomedical Engineering Department in the University of Barcelona.*

Prof. Samitier is President of the Catalan Association of Research Centres (Associació Catalana d'Entitats de Recerca - ACER) and member of the Institut d'Estudis Catalans (IEC, Catalan Academia). He was founder and member of supervisory board of EIT-Health, founder and coordinator of the Spanish Platform on Nanomedicine, member of the international committee of the International Society for BioMEMS and Nanotechnology, on the editorial board of the JOURNAL OF NANOSCIENCE AND NANOTECHNOLOGY, *Editor in chief of Biomimetics journal, and member of the international scientific committee of ITAV (Toulouse) and Cancéropole CLARA (Lyon). He has also been scientific advisor for a programme of the government of Argentina to foster nanotechnologies among SMEs and promoted and coordinated the Catalan Nanobiomedicine Alliance, involving 20 institutions in the Barcelona region.*

Since 2013 he has served as Spanish Delegate of the working group on Biotechnology and Nanotechnology of the Organization for Economic Cooperation and Development.

From March 2001 to June 2005 Prof. Samitier was Deputy Head of the Barcelona Science Park (PCB). From 2009 to 2013 he was one of the 20 Spanish researchers in biomedicine and biotechnology supported by the TechTransfer Programme of the Botin Foundation.

He has more than 260 ISI publications, with over 6000 citations and an H-index of 37. He received the Barcelona City Prize of Technology in 2003 and is inventor of 4 licensed patents.

His research focuses on the design and development of miniaturized devices containing micro- and nanoscale features for biomedical applications. His group develops microfluidics and lab-on-a-chip devices with integrated multiplexed electrochemical biosensors.

In 2020 he has been awarded by the Catalan Government with the Narcís Monturiol medal for his contribution to science and technology.

Tuesday 27th October · 10:00

Round table

Clinical translation of 3D in vitro and organoids systems to model diseases and accelerate drug development

Raquel Yotti

Raquel Yotti is the current Director General of the Spanish Institute of Health "Carlos III", the main public funding organization for biomedical research in Spain. She is a clinical cardiologist and until her appointment in September 2018, she was the head of the Clinical Cardiology Department at the Gregorio Maranon General University Hospital, and associate professor of the Department of Bio-engineering and Aerospace Engineering at the Carlos III University in Madrid. She is an expert in cardiac imaging and inherited heart diseases.

Maurice Whelan

Prof. Maurice Whelan is head of the Chemical Safety and Alternative Methods Unit of the Directorate for Health, Consumers and Reference Materials of the European Commission's Joint Research Centre (JRC), based in Ispra, Italy. He also heads the JRC's EU Reference Laboratory for alternatives to animal testing (EURL ECVAM). Maurice is the EU co-chair of the OECD Advisory Group on Molecular Screening and Toxicogenomics that is responsible for the OECD programme on Adverse Outcome Pathways; a member of the Steering Committee of the European Partnership for Alternative Approaches to Animal Testing (EPAA); and chair of the newly created Regulatory Advisory Board of the European Organ-on-Chip Society (EUROoCS). His publications include over 200 scientific papers and a recent book on the validation of alternative methods for toxicity testing. He has held a number of external appointments including the 2017-2018 Francqui Chair for alternative methods at the Vrije Universiteit Brussel (VUB, Belgium) and is currently visiting Professor of Bioengineering at the University of Liverpool (UK).

Anton Ussi

Anton Ussi (M) is the Operations and Finance Director at EATRIS C&S. Anton joined EATRIS in 2011 as Head of Operations and has been EATRIS Operations and Finance Director since 2015. He has a background in mechanical engineering in the automotive industry, spent several years as an SME entrepreneur, with several subsequent years undertaking university technology transfer in biomedical sciences, with a focus on medical and advanced imaging technologies. Anton also has a Master's degree in International Business Administration. He is a specialist in the establishment of public-private and public-public collaborations in the field of translational research in medicine, and has been co-responsible for the development of several ongoing partnerships and spin-out companies.

Wednesday 28th October · 15:00

Biomimetic materials in medicine and beyond

Javier Gómez Fernández

Nature has abundant materials with extraordinary mechanical characteristics and functionalities. Using the possibilities brought by recent advances in microelectronic engineering and biochemistry, we can reproduce, with unprecedented precision, the structural organization of natural environments and materials. As a result, we can harvest the principles of biological materials' designs to produce biologically-inspired composites with outstanding characteristics. We use these materials to tackle some of the current environmental challenges and produce medical devices. In this talk, we will cover the basic concepts of bioinspired materials and our latest developments in this exciting field of science and engineering.

Javier Gómez Fernández

Javier's research at SUTD is focused on the broad study, development, and application of biological materials in science and technology. In 2014 he was awarded the world's most outstanding young researcher in materials science by the Bayer Foundation. He has been also awarded with the Zwick Science Award for his studies on Mechanical Testing, and best PhD thesis at the University of Barcelona for studies in the use of biopolymers in Microelectronics and Biomedicine.

After his Ph.D, Javier moved to MIT, where he developed the "Micro-Masonry", a technology to assemble artificial organs and recognized as "breakthrough in tissue engineering" (CNET). Later, he moved to the Wyss Institute at Harvard University, where he designed and made "Shrilk", a compostable and biocompatible material inspired by the insect cuticle. Shrilk is referred, for example, to as "one of the materials that will change the future of manufacturing" (Scientific American), a "Supermaterial" (National Geographic), and has been chosen (with graphene) one of the "five material that could change the word" (The Guardian).

Surface control of adhesion and growth factor mechanical and chemical crosstalk

E. Ada Cavalcanti-Adam

Max Planck Institute for Medical Research; Heidelberg, Germany

Mechanical and chemical cues present in the extracellular environment regulate cell adhesion-mediated responses, such as migration, proliferation and differentiation. To address how local changes in the extracellular environment regulate cell responses through specific receptor-ligand interactions material surfaces can be designed to combine adhesive ligands and growth factors at the nanoscale. I will present surface functionalization strategies to control integrin clustering and cellular adhesion forces. The nanoscale presentation of integrin ligands is also combined with growth factors, namely bone morphogenetic proteins (BMPs), to modulate cell osteodifferentiation. In this talk, I will also discuss the synergistic effect of BMPs and mechanical cues on osteogenic signaling and mechanotransduction, elucidating the interdependency of Smad 1/5 and YAP/TAZ signaling.

E. Ada Cavalcanti-Adam

Ada Cavalcanti is Departmental Group Leader at the Max Planck Institute for Medical Research and Member of the Faculty of Biosciences at the Heidelberg University. Her research is centered on the mechanobiology of cell-matrix adhesion. She combines surface patterning and functionalization approaches with biophysical techniques to study the nanoscale regulation of cell adhesion structures and signaling. She has authored >60 publications in international peer-reviewed journals. Her research is supported by the Max Planck Society and by the German Science Foundation (DFG) and she has been awarded in 2008 with the prize "for women in science" from UNESCO-L'Orèal.

Wednesday 28th October · 16:40

Microtechnologies and nanotechnologies in Drug Delivery

Robert S. Langer
Institute Professor
Massachusetts Institute of Technology

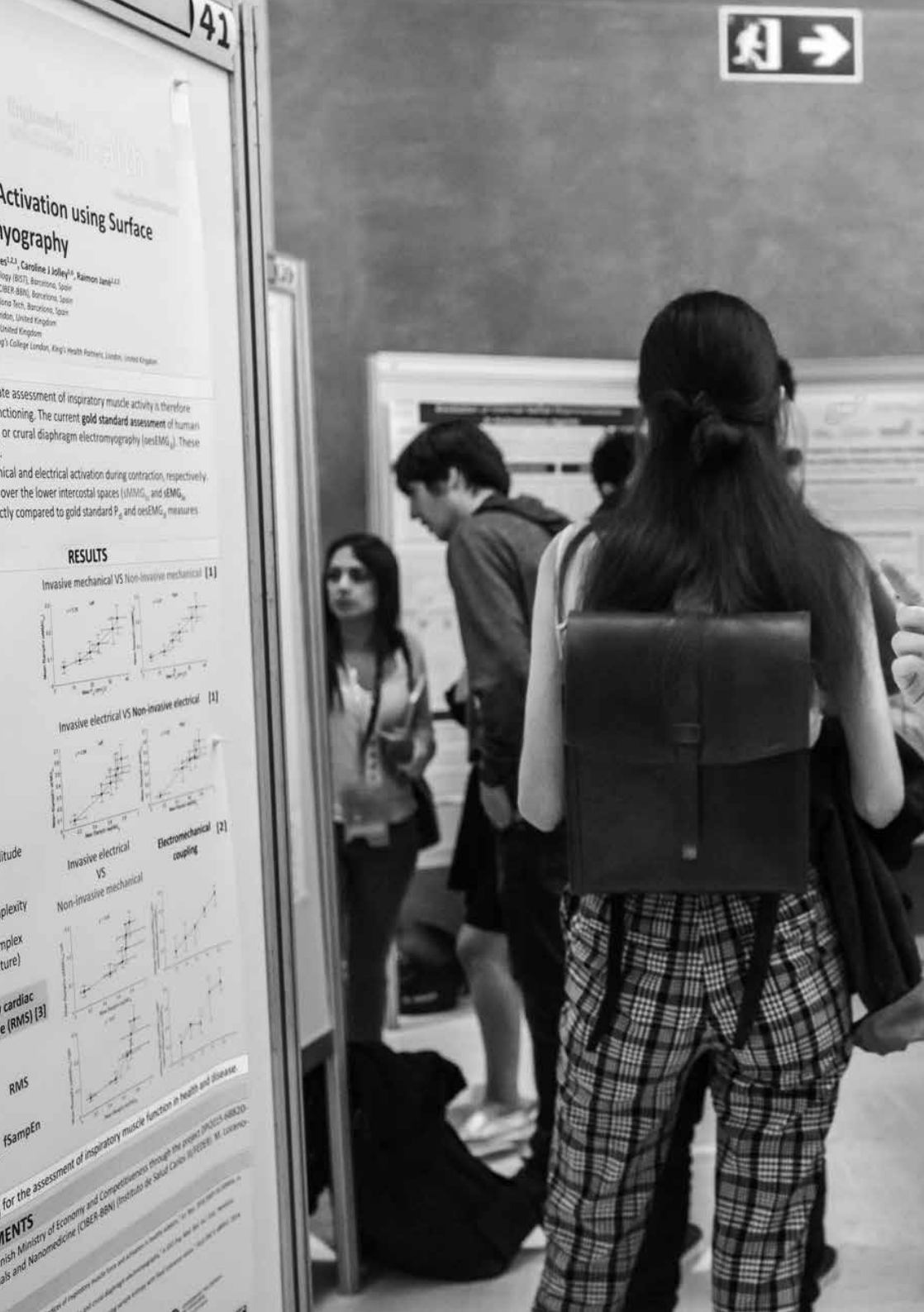
There are numerous new technologies being developed that may impact the future of medicine. For example, new drug delivery technologies including microparticles, nanoparticles and nanotechnology promise to create new treatments for cancer, heart disease and other illnesses. Nanotechnology may also be useful in delivering DNA and siRNA as well. Approaches involving polymers, microchips, and lipids will be examined.

Robert Langer

Robert S. Langer is one of 12 Institute Professors at MIT; being an Institute Professor is the highest honor that can be awarded to a faculty member. Dr. Langer has written more than 1,500 articles. He also has over 1,400 issued and pending patents worldwide. Dr. Langer's patents have been licensed or sublicensed to over 400 pharmaceutical, chemical, biotechnology and medical device companies. He is the most cited engineer in history (h-index 279 with over 321,000 citations according to Google Scholar).

He served as a member of the United States Food and Drug Administration's SCIENCE Board, the FDA's highest advisory board, from 1995 — 2002 and as its Chairman from 1999-2002.

Dr. Langer has received over 220 major awards. He is one of 4 living individuals to have received both the United States National Medal of Science (2006) and the United States National Medal of Technology and Innovation (2011). He also received the 1996 Gairdner Foundation International Award, the 2002 Charles Stark Draper Prize, considered the equivalent of the Nobel Prize for engineers, the 2008 Millennium Prize, the world's largest technology prize, the 2012 Priestley Medal, the highest award of the American Chemical Society, the 2013 Wolf Prize in Chemistry, the 2014 Breakthrough Prize in Life Sciences and the 2014 Kyoto Prize. In 2015, Dr. Langer received the Queen Elizabeth Prize for Engineering. Among numerous other awards Langer has received are the Dickson Prize for Science (2002), the Heinz Award for Technology, Economy and Employment (2003), the Harvey Prize (2003), the John Fritz Award (2003) (given previously to inventors such as Thomas Edison and Orville Wright), the General Motors Kettering Prize for Cancer Research (2004), the Dan David Prize in Materials Science (2005), the Albany Medical Center Prize in Medicine and Biomedical Research (2005), the largest prize in the U.S. for medical research, induction into the National Inventors Hall of Fame (2006), the Max Planck Research Award (2008), the Prince of Asturias Award for Technical and Scientific Research (2008), the Warren Alpert Foundation Prize (2011), the Terumo International Prize (2012), the Benjamin Franklin Medal in Life Science (2016), the Kabiller Prize in Nanoscience and Nanomedicine (2017) and the Dreyfus Prize in Chemical Science (2019). In 1998, he received the Lemelson-MIT prize, the world's largest prize for invention for being "one of history's most prolific inventors in medicine." In 1989 Dr. Langer was elected to the National Academy of Medicine, in 1992 he was elected to both the National Academy of Engineering and to the National Academy of Sciences, and in 2012 he was elected to the National Academy of Inventors.


41
Activation using Surface
nyography
Caroline J Jolley
RESULTS
Invasive mechanical VS Non-invasive mechanical [1]
Invasive electrical VS Non-invasive electrical [1]
Invasive electrical VS Non-invasive mechanical
Electromechanical coupling [2]
RMS
fSampEn
for the assessment of inspiratory muscle function in health and disease.

Posters and flash presentations

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ICT FOR HEALTH - Posters with flash presentation

Poster	Name	Title
1	Ignasi Ferrer	Home Sleep Apnea Monitoring: Analysis of Smartphone Triaxial Accelerometry for Assessing Disordered Breathing and Sleep Position

ICT FOR HEALTH - Posters

Poster	Name	Title
2	Gabriele Marchello	High-resolution 4D imaging of soft matter in liquid water
3	Dolores Blanco-Almazán	Bioimpedance and Surface Myographic Signals for Noninvasive Assessment of COPD during Loaded Breathing
4	Alexandre Brandao	KinesiOS: Software for Measuring Range of Motion and Recording Data
5	Yolanda Castillo Escario	Electromyography and Smartphone Accelerometry to Study Trunk Flexion during Reaching in Healthy Human Subjects
6	Clare Davidson	Spatial Distribution of Normal Lung Sounds during Varied Inspiratory Load and Flow Conditions
7	Luis Carlos Estrada Petrocelli	Evaluation of surface diaphragm electromyography using concentric ring electrodes

8	Luis Fernandez	Feature extraction strategies for GC-IMS metabolomics: A systematic approach.
9	Adrián Fernandez	Cholinergic control of chaos and evidence sensitivity in a neocortical model of perceptual decision-making
10	Manuel Lozano García	Noninvasive Assessment of Inspiratory Muscle Neuromechanical Coupling using Surface Mechanomyography and Electromyography
11	Ceclia Mallafré Muro	Design of experiment for optimization of volatiles compounds extraction in urine analysis
12	Fabian Obstals	Outstanding sensitivity and selectivity in biomarker detection for medical diagnostics
13	Luciana Oliveira	Signal Processing for Gas Chromatography - Mass Spectrometry: Evaluation of compounds related with exacerbation on Chronic Obstructive Pulmonary Diseases (COPD)
14	Daniel Romero	Combining HRV and pulmonary function related markers to explain cardiac comorbidities in COPD patients
15	Andreu Vaquer	Mobile wearable biosensors with enzyme-modulated dynamic range for the simultaneous detection of sweat lactate and sweat volume

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NANOMEDICINE - Posters with flash presentation

Poster	Name	Title
16	Maria Arista Romero	Nanostructural characterization of influenza virus-like particles with super resolution microscopy
17	Elena Lantero	Heparin Administered to Anopheles in Membrane Feeding Assays Blocks Plasmodium Development in the Mosquito
18	Maximilian Loeck	Role of Valency on the Ability of ICAM-1-Targeted Nanoparticles to Effectively Cross the Blood-Brain Barrier and Deliver Therapeutic Enzymes in Cellular and Animal Models
19	Albert Manzano	Personalizing pediatric cancer treatment with dynamic BH3 profiling
20	Laura Moya	A clearing protocol for Galleria mellonella larvae: Visualization of internalized fluorescent nanoparticles
21	Davia Prischich	In vivo photocontrol of adrenergic neurotransmission
22	Mireia Seuma	Using genomics to map amyloid nucleation in Alzheimer's disease
23	Shubham Tanwar	Nanoscale Mapping of the Conductivity and Interfacial Capacitance of an Electrolyte Gated Organic Field Effect Transistor under Operation

NANOMEDICINE - Posters

Poster	Name	Title
24	Cristina Adrover	Mobile Paper Biosensors for Screening Elevated Cytokine Levels in Blood Samples from COVID-19 Patients
25	Enrico Almici	Identification of new collagen-associated biomarkers in lung cancer by advanced image analysis of patient biopsies
26	Teodora Andrian	Correlating super-resolution microscopy and electron microscopy to study size and ligand heterogeneity in nanoparticles
27	Azzurra Apriceno	Design of multivalent polymersomes for range-selective binding
28	Marta Aranda Palomer	An injectable nanobiosensor for continuous remote monitoring of cancer patients
29	Xavier Arqué	Improving self-propulsion of urease-powered micromotors
30	Yunuen Avalos Padilla	The ESCRT-III machinery from Plasmodium falciparum participates in the biogenesis of extracellular vesicles during infection
31	Harishankar Balakrishnan	On the Spatial Resolution of Nanotomography Based on Scanning Dielectric Microscopy: a Numerical Analysis
32	Inés Bouzón Arnáiz	Targeting protein aggregation in Plasmodium falciparum as a new antimalarial design strategy.
33	Nuria Camarero	Photoswitchable dynasore analogs to control endocytosis with light
34	Mar Cendra	Differential adaptability between reference strains and clinical isolates of Pseudomonas aeruginosa into the lung epithelium intracellular lifestyle

35	Claudia Di Guglielmo	Polymersomes eradicating Tuberculosis
36	Martina Di Muzio	Lamellarity and Electrical Properties of Single Liposomes Measured by In-Liquid Scanning Dielectric Microscopy
37	Beatriu Domingo Tafalla	Mechanical and structural characterization of Quatsome vesicles by AFM and X-Ray techniques.
38	Adrianna Glinkowska	Microfluidic technology for real-time imaging of drug delivery systems stability and extravasation
39	Manuela Garay-Sarmiento	Antimicrobial and non-adhesive electrospun wound dressings of polycaprolactone
40	Coral García Fernández	Novel mRNA vaccination strategies: use of poly(beta aminoesters) to achieve selective dendritic cells delivery
41	Daniel Gonzalez Carter	Targeting nanoparticles to the brain by exploiting the blood–brain barrier impermeability to selectively label the brain endothelium
42	Ana C. Hortelão	Nanobots Towards Bladder Cancer Theranostics: From In vitro Targeting to In Vivo Imaging
43	Larissa Huetter	Multiscale Modeling of Organic Electronic Biosensor Response
44	Anton Joseph	First steps towards mimicking cell division in fully artificial systems
45	Nina Kostina	Building membrane machines to endocytize living bacteria: the battle between adhesion and flexibility
46	Anna Lagunas	Electron Transport Study in Olfactory Receptor 1A1
47	Sonia Lanzalaco	Study of a New Generation of Surgical Mesh for hernia repair: a flexible, smart and self-evolving sensor/actuator with 4D Response
48	Manuel López Ortiz	Unidirectional Photosynthetic Complex functionalization for tunnel current distance decay spectroscopy
49	Galyna Maleeva	Azobenzene/nitrazepam-based light-controllable modulators of inhibitory brain receptors

50	Yannick Yannick	Anti-fouling hydrogel coatings for medical devices
51	Ruben Millan-Solsona	Mapping the Capacitance of Self-Assembled Monolayers at Metal/Electrolyte Interfaces at the Nanoscale by In-liquid Scanning Dielectric Microscopy
52	Madhura Murar	Multi-ligand targeting nanoparticles for personalized cancer therapy
53	Rodica Alis Olea	Judging micelles by their covers: combining molecular precision and self-reporting mechanism for a direct comparison of dendritic amphiphiles with different hydrophilic blocks
54	Sujey Palma	Development and permeability evaluation through BBB-on-a-chip model of Gold nanorods with therapeutic potential for Alzheimer's disease
55	Jonas Quandt	Controlling the Activation of Coagulation at Interfaces
56	Carlota Roca Martinez	Development of DNA aptamers against enzymes of the methylerythritol phosphate pathway
57	Lucia Roman	Modified phospholipid vesicles as drug delivery systems for the treatment of leishmaniasis
59	Jana Simon Campreciós	Study of the relative expression of ICAM-1 Ig-like domains in different cell types and disease conditions
60	Dominik Soeder	Enhanced binding by hierarchical self-assembly of sugars in glycodendrimersomes
61	Rosalba Sortino	Rational design of photochromic analogs of tricyclic drugs
62	Jan Tenbusch	Super-flexible Biomimetic Vesicles Kill E. coli
63	Mariia Vorobii	Polymer Brushes as a Platform to Improve Biocompatibility of the Coatings
64	Ana Maria Wagner	Unravelling topology-induced shape transformations in dendrimersomes
65	Lena Witzdam	Fibrinolytic Coating System to Achieve Adaptive Hemocompatibility of Blood-Contacting Devices

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MECHANOBIOLOGY - Posters with flash presentation

Poster	Name	Title
66	Jenny Zanetta Kechagia	Laminin impairs mechanosensing by protecting the nucleus from mechanical loading.
67	Ana López Mengual	Molecular and physical factors modulating Cajal-Retzius cells migration and distribution in cortical development
68	Macià Esteve Pallares Pallares	Ultrafast cadherin durotaxis through a wetting transition

MECHANOBIOLOGY - Posters

Poster	Name	Title
69	Annalisa Calo	Spatial Mapping of the Collagen Distribution in Human and Mouse Tissues by Force Volume Atomic Force Microscopy
70	Gerardo Ceada Torres	Mechanical Compartmentalization of the Intestinal Organoid Enables Crypt Folding and Collective Cell Migration
71	Nimesh Chahare	Microfluidic device for engineering 3D epithelial monolayers with controlled pressure
72	Natalia Díaz Valdivia	Stromal SMAD3 enhances collective cancer cell migration and invasion led by fibroblasts in 3D culture models
73	Laura Faure	New system to study single cell mechanoresponse in 3D

74	Marta Gabasa Ferrández	Interleukin-1β decreases contractility and attenuates migration and wound-repair ability in primary human pulmonary fibroblasts
75	Ignasi Granero-Moya	Mechanosensitive control of nucleocytoplasmic transport
76	Tamás Haraszti	Imaging based determination of mechanical properties of vesicles and synthetic cells
77	Anabel-Lise Le Roux	Dynamic mechanochemical feedback between curved membranes and BAR protein self-organization
78	Ariadna Marín Llauradó	Linking epithelial geometry to tension and pressure
79	Jorge Otero	Low-cost device to measure maximal inspiratory (MIP) and expiratory (MEP) pressures
80	Jorge Otero	Low-cost open-source pressure support ventilator for patients with respiratory failure
81	Xarxa Quiroga Álvarez	IRSp53 orchestrates local actin polymerization to remodel plasma membrane upon stretch
82	Alvaro Villarino	Cyclic stretching mesenchymal stromal cells cultured on lung hydrogels
83	Ian Williams	Diffusioosmotic and convective flows induced by a non-electrolyte concentration gradient

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CELL ENGINEERING - Posters with flash presentation

Poster	Name	Title
84	Maria Guix Noguera	Smart skeletons for 3D printed living biobots
85	Enara Larrañaga Carricajo	Cell-substrate adhesion drives intestinal epithelial cell organization
86	Lluís Oliver Cervelló	The Capacity of RGD and DWIVA Peptidic Biointerface to Transdifferenciate C2C12 Myoblasts into Osteogenic Lineage
87	Adrián López Canosa	Microfluidic System to Mimic the Bone Healing Microenvironment and Study the Role of Calcium in Endothelial Progenitor Cell Recruitment
88	Idoia Lucia Selfa Aspiroz	Studying Wilms' Tumor 1 (WT1) function in human kidney development and disease using human pluripotent stem cells-derived organoids and genome editing
89	Jose Yeste Lozano	In situ Metabolomics in 3D cell cultures in Organ-On-Chips by hyperpolarization-enhanced Magnetic Resonance

CELL ENGINEERING - Posters

Poster	Name	Title
90	Aina Abad	Microengineered Villus-Like PEGDA Hydrogels Under Spatio-Biochemical Gradients For Primary Intestinal Epithelium In Vitro Model
91	Marc Azagra	Towards a non-invasive biomarker of myotonic dystrophy 1 using NMR-based assays of muscle cells

92	Barbara Blanco Fernandez	Development of a breast cancer model based on elastin-like recombinamers for drug discovery
94	Maria Gallo	Generation of human and pig kidney decellularized extracellular matrix: towards the fabrication of kidney-specific bio-inks for 3D Bioprinting applications
95	Carmen Hurtado del Pozo	Erasing metabolic alterations in proximal tubular cells under hyperglycaemic condition using inducible CRISPR/ Cas9 PGC1a hESC-derived 3D kidney organoids
96	Laia Lidón Gil	PrPC regulates alternative splicing of tau by modulating GSK3β activity
97	Gerard Rubí-Sans	Development of a drug screening model using 3D cell-derived extracellular matrices
98	Veronika Magdanz	Flagellated flexible magnetic microrobots
99	Andrés Marco Giménez	Engineering human Pluripotent Stem Cells (hPSCs) lines with CRISPR/Cas9 for constitutive and inducible Knock Out in Kidney Organoids.
100	Sara Martínez Torres	Peripheral cannabinoid type 1 receptor blockade enhances memory persistence in mice through an adrenergic mechanis
101	Marina Martínez-Hernández	Promoting in situ cardiac regeneration through lactate-based biomaterials
102	Francina Mesquida-Veny	Characterization of the intrinsic changes induced by the modulation of neuronal activity after central nervous system injury
103	Sara Morini	Biomanufacturing platform development for the large-scale production of non-parenchymal cells towards the bioengineering of a porcine whole liver
104	Jorge Otero	3D Bioprinting lung resident mesenchymal stromal cells in extracellular matrix hydrogels
105	Carmen Hurtado del Pozo	Study of the interplay between glucose metabolism and SARS-CoV-2 infection in kidney organoids
106	Mehrnoush Rahimzadeh	Combisomes Solve the Dilemma of Polymer-based Cell-Mimetic Membranes
107	Sergi Rey Viñolas	Personalized Bioactive and Biodegradable Implants for Maxillofacial Bone Regeneration
108	Celia Ximenes-Carballo	Development of ion-releasing platforms for wound healing applications

Home Sleep Apnea Monitoring: Analysis of Smartphone Triaxial Accelerometry for Assessing Disordered Breathing and Sleep Position

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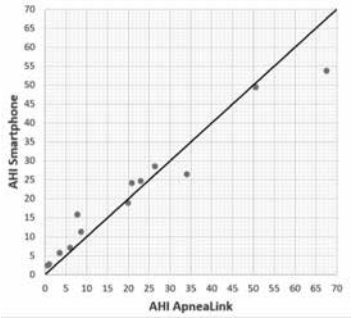
Obstructive sleep apnea (OSA) is a sleep disorder in which repetitive events of total or partial upper airway occlusion take place during sleep. This occlusion is responsible for a reduction of the respiratory flow that induces the appearance of periods of hypoxia, which is known to be an important risk factor for multiple cardiovascular and cerebrovascular diseases. A significant burden is that OSA, even though is a common disorder estimated to affect 7-21% of adult men and 2-11% of adult women [1], has a low diagnosis ratio, leaving around 75-80% of patients undiagnosed and untreated [2]. In addition, OSA is also known to be position-dependent in some patients, which is referred to as positional OSA (pOSA). The development of tools to monitor OSA and pOSA at home [3] is of great interest, since more personalized and effective treatment strategies are required. In this work, we analyzed the triaxial accelerometry obtained from a smartphone placed over the sternum to detect sleep disordered breathing and sleep position. We developed an algorithm which identified disordered breathing events, linked to OSA, and determined the likelihood of a patient to suffer from pOSA. The validation of the smartphone system was performed by comparing the events found by our system with the ones from a portable commercial OSA diagnosis device (Apnealink) at home; we also validated the sleep position determined by our system with the position provided by a gold-standard polysomnographic (PSG) system. The results of the event comparison can be seen in Figure 1, where the apnea hypopnea index (AHI) obtained for each subject analyzed was very similar between both devices. In Figure 2, it is also possible to see an example of the sleep position retrieved by the smartphone and the PSG, where the agreement between both devices is also very high. These results indicate that smartphones are promising mHealth tools to be used for OSA and pOSA assessment at home.



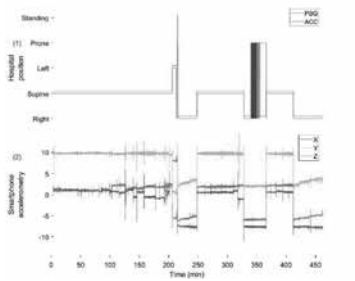


Figure 1: Comparison between the AHI obtained from the smartphone event detector and the AHI from the reference device Apnealink for 13 subjects.

Figure 2: Example of the correlation between the sleep position signal obtained by the smartphone and the sleep position signal obtained from the PSG.

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High-resolution 4D imaging of soft matter in liquid water

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Electron microscopy (EM) is a technique that exploits the interaction between electron and matter to produce high resolution images down to atomic level. In order to avoid undesired scattering in the electron path, EM samples are conventionally imaged in solid state under vacuum conditions. Recently, this limit has been overcome by the realization of liquid-phase electron microscopy (LP EM), a technique that enables the analysis of samples in their liquid native state. LP EM paired with a high frame rate acquisition direct detection camera allows tracking the motion of particles in liquids, as well as their temporal dynamic processes. In the present work, soft organic and biological materials were imaged in their liquid native state and their structural assemblies were reconstructed. However, the choice of organic soft material made the task of imaging very arduous, due to poor contrast.

The study presented here aims to take advantage of particle motion screened in videos, to overcome one of the main limits of the single particle analysis (SPA) method. SPA is a well-known technique that uses images of thousands of particles of the same nature, to reconstruct 3D models of the particle itself. Images showing different profiles of the particle are clustered according to a similarity criterion, and put together, but problems may arise when angular orientations have to be assigned. Therefore, to solve this problem a priori model can be used to facilitate the orientation assignments. The main drawback is the goodness of the *a priori* model inevitability biases the final result of the reconstruction. We have overcome this drawback by merging single particle analysis techniques with the dynamics of particles. The images to process do not contain hundreds of particles, as conventionally requested by the SPA technique, but in this case, the high variety of particle profiles is produced by the Brownian motion associated with the liquid state. In this method – thus named Brownian Particle Analysis (BPA), time becomes a pivotal component of the reconstruction process: particles picking is performed along different frames, rather than across a single frame. The involvement of time in the reconstruction extends

the algorithm into a third dimension that conventionally does not exist. Consequently, the high variety of particles profiles that the reconstruction algorithm demands are provided by recording the motion of particles in time.

In this scenario, the postprocessing phase assumes more and more importance inside the general algorithm, since sharpening single images can lead to uncover very specific features, useful to track the direction of the rotations. Furthermore, this project eliminates any boundaries to *a priori* knowledge and can be applied to a very wide range of particles, with an emphasis in soft material nanoparticles and biomolecules.

Bioimpedance and Surface Myographic Signals for Noninvasive Assessment of COPD during Loaded Breathing

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Chronic Obstructive Pulmonary Disease (COPD) is a progressive and common disease in the adult population that is characterized by the airflow limitation of the patients. COPD is a major cause of chronic morbidity and mortality representing an important socioeconomic burden worldwide [1]. The current assessment of COPD requires a maximal maneuver during a spirometry test to quantify the airflow limitation. Other less invasive measurements such as thoracic bioimpedance and surface myographic signals have been studied as an alternative to classical methods to provide relevant information about respiration. Particularly, previous studies reported the linear relationship between thoracic bioimpedance and respiratory volume [2], [3] and suggested that respiratory volume is the main contribution to thoracic bioimpedance measurement [4]. On the other hand, strong correlations have been shown between surface myographic signals and the invasive measures of inspiratory muscle force [5]. The main objective of this study is to investigate bioimpedance and its combination with surface myographic parameters in COPD patients to assess the applicability of these novel noninvasive measures in respiratory disease monitoring [6]. We measured bioimpedance, surface electromyography and surface mechanomyography in forty-six COPD patients during an incremental inspiratory threshold loading protocol. We proposed two novel features computed as the change of bioimpedance over the electrical and mechanical activity for each respiratory cycle. These features demonstrate significant differences between mild and severe patients, indicating that the severest COPD patients had lower inspiratory contribution of the inspiratory muscles to global ventilation. In conclusion, the combination of bioimpedance and surface myographic signals provides useful indices for the noninvasive assessment of the COPD condition.

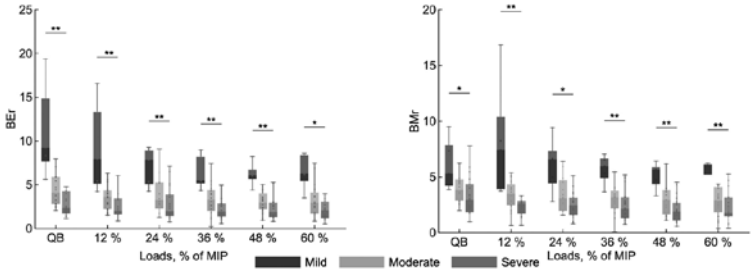

Figure 1 Ratios computed from thoracic bioimpedance, surface electromyography and surface mechanomyography signals of the three severity groups over the quiet breathing and the different inspiratory load levels. * denotes p-value < 0.05 and ** denotes p-value < 0.01

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KinesiOS: Software for Measuring Range of Motion and Recording Data

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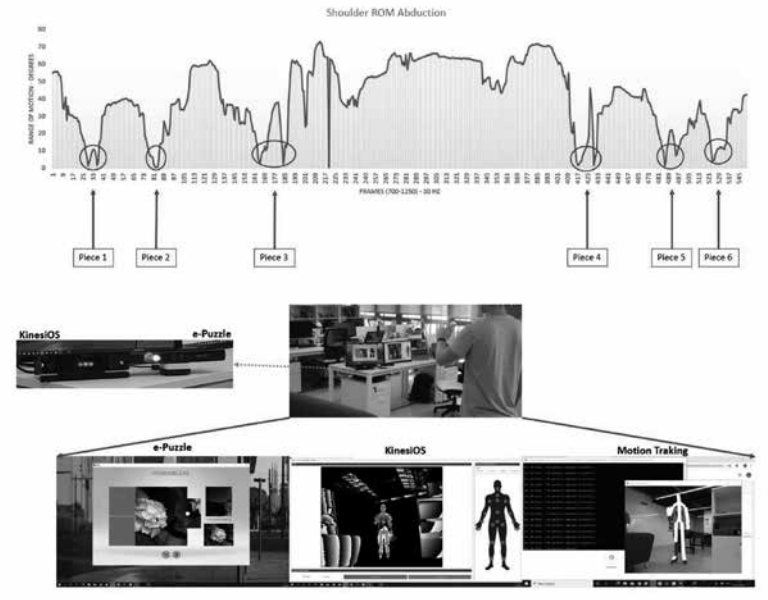
The study of the angular movement of the human body requires kinematic parameters, such as the range of motion (ROM). Motor coordination of an individual allows precise movements mainly through a dynamic (isotonic) contraction, in which the force developed by the muscle is greater or less than the resistance. This situation enables control of movement against gravity (concentric contraction) or in favour of gravity (eccentric muscle action). The proposed software KinesiOS, that uses the Kinect sensor, offers an option to health experts that study human movement (kinesiology) and represents a low-cost alternative being a non-invasive assessment resource. The e-Puzzle application, which is also responsive to the gesture recognition sensor (Kinect device), consists of a puzzle game in which the fitting of the pieces occurs according to the user's gestures. In this case, the spatial coordinates of the user's hand are processed and converted into a command for the e-Puzzle application so that the user can control the puzzle game from gestural interaction. On the left side of the interface, that is a grid to fit the pieces, and on the right part of the interface, there are puzzle pieces scrambled. To assemble the puzzle, the user must take one of the pieces and explore the matrix until the piece fits in the correct place. Thus, it is possible to explore the movements of the upper limbs in all planes and range of motion (coronal, sagittal and transverse), allowing control both from the orthostatic position and from the sitting position. If the user presents a limitation of movement of the upper limbs (like in a rehabilitation process), it is possible to decrease the range of motion by approaching the sensor. Hence, less movement is necessary to move the pieces from one side to the other of the interface. This version of the KinesiOS software should the acquisition of the ROM data in the coronal and sagittal plane (figure), and the next step will be to synchronize more than one sensor to improve the accuracy of the data collected in the sagittal plane and allow the collection of data in the transverse plane.


Shoulder ROM Abduction
Piece 1
Piece 2
Piece 3
Piece 4
Piece 5
Piece 6
KinesiOS
e-Puzzle
e-Puzzle
KinesiOS
Motion Traking

Electromyography and Smartphone Accelerometry to Study Trunk Flexion during Reaching in Healthy Human Subjects

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Trunk stability is essential to maintain upright posture and support functional movements, but there is need for quantitative measures for trunk evaluation. In this study, we aimed to 1) characterize the muscle activity and movement patterns of trunk flexion during an arm reaching task in sitting healthy subjects, and 2) investigate whether trunk stability is affected by a startling acoustic stimulus (SAS). For these purposes, we recorded the electromyographic (EMG) activity of 8 muscles (from trunk, neck, and shoulder) and the smartphone accelerometer signals in 33 healthy volunteers during a trunk bending task. We analyzed these signals to extract multiple features, including the EMG onset latencies and amplitude parameters of all muscles, and the tilt angle and movement features from smartphone accelerometer data. We also measured the reaction time, as the time to press the switch button from the go-signal. Two-way repeated measures ANOVAs were applied to examine the effects of SAS and target distance (15 cm vs. 30 cm).

The reaction time was significantly reduced in SAS vs. non-SAS trials: 554±75 ms vs. 650±85 ms at 15 cm, and 615±73 vs. 721±74 ms at 30 cm ($p<0.001$). The EMG onset latencies of all muscles, either prime movers or stabilizers, were also significantly shorter in SAS trials ($p<0.001$ for all muscles, Fig. 1). However, the SAS did not change neither muscle recruitment pattern nor movement duration ($p=0.41$). The accelerometer signals had a higher frequency content in SAS trials ($p<0.001$), suggesting reduced movement control. Regarding the distance effect, longer durations ($p<0.001$), higher tilt angles ($p<0.001$), and higher EMG amplitudes ($p<0.001$ for all muscles) were observed at 30 cm compared to 15 cm.

The proposed neurophysiological measures and mHealth tools have helped to establish the trunk flexion pattern in arm reaching in healthy subjects, which could be useful for future objective assessment of trunk stability in patients with neurological affections.

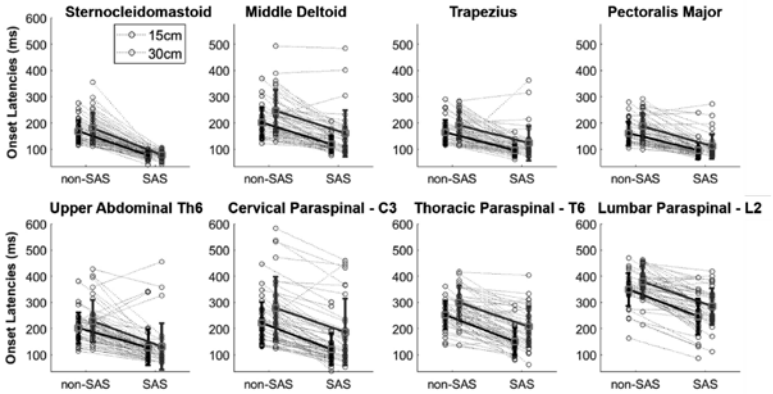

Figure 1. Onset latencies for the eight muscles and all the subjects, during the trunk movement task in non-SAS and SAS trials, at 15 cm (black) and 30 cm (blue). Each dotted line represents one subject, while the mean values are shown in wider lines. Error bars correspond to the standard deviation.

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Spatial Distribution of Normal Lung Sounds during Varied Inspiratory Load and Flow Conditions

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Respiratory sounds (RS) contain important information about the functioning of the respiratory system in both health and disease, and are commonly used by physicians to detect, assess and monitor respiratory conditions. However, in current clinical practice, RS are usually subjectively assessed using a stethoscope, which is limited by the expertise and hearing acuity of the health professional involved. Normal lung sound intensity (LSI) is a characteristic of RS that has been found to correlate well with airflow [1]. It can therefore be used to quantify airflow changes and limitations that can occur with respiratory disease.

In this study, the spatial distribution of normal LSI of a healthy population during two separate protocols is examined thereby permitting the exploration of a range of variations in breathing such as those physiologically employed in response to external stressors. LSI is usually assessed using the root mean square (RMS) method. In this study a novel representation of LSI using fixed sample entropy (fSampEn) is also explored. fSampEn is less sensitive to impulsive noise such as microphone clicks and cardiac activity than RMS [2].

Respiratory sounds and airflow were simultaneously recorded from 12 healthy individuals during two separate protocols; an inspiratory loading protocol which simulates different levels of effort while breathing, and a varying airflow protocol which mimics changes in the speed and volume of breathing. RS was recorded using four piezoelectric contact microphones (TSD108, Biopac Systems, Inc.) placed on the posterior skin surface of the subject. Airflow was acquired with a pneumotachograph (4830; Hans Rudolph Inc., KS, USA). A total of 100 inspiratory cycles, lying as close to the mean inspiratory cycle for that subject and protocol level, were selected for each subject and used to examine the spatial distribution of the LSI. Further details of the experimental protocol can be found in [3], [4].

During the protocols explored here the normal LSI in the left and right lungs, upper and lower, in healthy populations was found to be similar, with asymmetries of less

than 3 dB, agreeing with values reported in other studies [5]. The fixed sample entropy of the RS signal was also calculated and found to compare favorably with the gold standard RMS representation of LSI. The results obtained contribute to the current knowledge of RS in healthy populations and can be used as a basis upon which to assess the response of patient populations to similar protocols.

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Evaluation of surface diaphragm electromyography using concentric ring electrodes

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Respiratory function test provides information about clinical condition of individuals with suspected or diagnosed respiratory disease. Measurements of respiratory volume, airflow and pressure reflect the muscle respiratory load on the respiratory system. Electromyography of the diaphragm (EMGdi), the primary muscle involved in respiration, is a surrogate measure of the neural respiratory drive that allows the study of the load and capacity imbalance on the respiratory muscles. However, due to its invasiveness and sophistication, the EMGdi technique results impractical for use in many medical applications. To overcome this limitation, a non-invasive approach for EMGdi recordings based on the use of surface electrodes (sEMGdi) has attracted attention for use as a clinical tool. Commonly, sEMGdi can be easily obtained using surface electrodes in bipolar configuration. Although the use of sEMGdi is advantageous for the evaluation of respiratory muscle function, its recording depends on the muscle fibers orientation as well as being influenced by electrocardiographic (ECG) activity, which compromises its analysis [1]. To alleviate the disadvantage of using the bipolar configuration, this study proposes the use of a concentric ring electrode (CRE) for sEMGdi acquisition [2].

The aim of the present study was to evaluate the performance of CRE during sEMGdi acquisitions and to compare their measurements with those obtained using the bipolar configuration. Thirty- two healthy adults (16 male), 23.5±2.9 years, 1.70±0.08 m, weight of 63.8±11.8 kg, and 21.9±2.7 kg/m2 participated in the study. sEMGdi was recorded using a pair of electrodes in bipolar configuration and a CRE placed bilaterally on the lower chest (Fig. 1) along with the inspiratory mouth pressure. The protocol consisted of a breath hold maneuver (BHM) and an incremental inspiratory threshold loading at 0%, and at 20, 40 and 60% of maximum inspiratory mouth pressure [2]. The protocol was performed twice switching the electrodes. Fig. 2 shows the combined (left and right) distribution of spectral power of inspiratory segments without ECG (P_{INC}) normalized to maximal P_{INC} ($P_{INC\%max}$) and the ratio P_{INC} to power of inspiratory segment with ECG during the BHM ($R_{ins/cardio}$).

The greater the respiratory load, the greater the $P_{INC\%max}$ and the $R_{ins/cardio}$ ratio. $R_{ins/cardio}$ of the CRE was greater than that of the bipolar configuration. In conclusion, CREs have great potential for improving the evaluation of respiratory muscle activity. $sEMG_{di}$ recordings can benefit from the advantages that the CRE has over the bipolar configuration, such as the reduced influence ECG and the electrode orientation problem.

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This work was supported in part by the Generalitat de Catalunya (CERCA Programme and GRC 2017 SGR 01770), the Gobierno de España (RTI2018-098472-B-I00 MCIU/AEI/FEDER, UE), and the Biomedical Research Networking Centre in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN, Instituto de Salud Carlos III/FEDER).

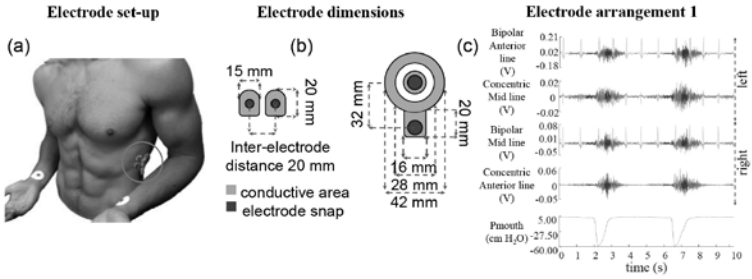

Figure 1. (a) Electrode set-up for respiratory sEMGdi signal acquisition corresponding to the first electrode arrangement. (b) Dimensions of surface electrode in bipolar configuration and a concentric ring electrode. (c) Representative sEMGdi cycles during 20% of maximum inspiratory mouth pressure recorded using bipolar and concentric ring electrodes on the left and right side of the chest.

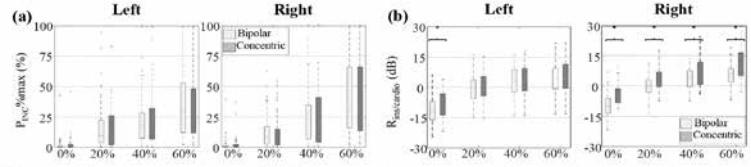

Figure 2. (a) Spectral power of inspiratory sEMGdi segments without cardiac activity (PINC) normalized to maximal PINC (PINC%max) during maximal inspiratory maneuver for the different levels of inspiratory loads of electrode configurations (bipolar, concentric) recorded on the lower left and right side of the chest. (b) Power ratio of inspiratory sEMGdi in relation to the power of basal sEMGdi with ECG (Rins/cardio) during breath hold maneuver for the different levels of inspiratory loads of electrode configurations (bipolar, concentric) recorded on the left and right side of the chest. Statistically significant differences ($p<0.05$) between electrode configurations are shown (*). Friedman's non-parametric test was used to assess significant differences.

Feature Extraction Strategies For GC-IMS Metabolomics: A Systematic Approach.

Luis Fernandez[1,2], Rafael Freire[1], Celia Mallafre[1,2], Luciana Oliveira[1], Francisco Madrid[1,3], Andrés Martín-Gómez[4], Lourdes Arce[4], Santiago Marco[1,2]

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Gas Chromatography – Ion Mobility Spectrometry (GC-IMS) allows fast, reliable and inexpensive chemical composition analysis of volatile mixtures. This sensing technology has been successfully employed in food science to determine food origin, freshness and preventing alimentary fraud. However, GC-IMS data is high dimensional, complex, and suffers from non- idealities that must be corrected to properly extract relevant features from samples. The feature extraction process in GC-IMS data is typically performed using peak picking and clustering methods, and the extracted features are arranged in a peak table format.

In this work, a pipeline for data pre-processing along with four different approaches for feature extraction in GC-IMS data are presented. More precisely, these approaches consisted in extracting data features from: 1) the area of the chromatogram for the Reactant Ion Peak (RIP), 2) the chromatogram for the RIP, 3) the whole sample matrix and, 4) a peak table. The resulting pipelines for data processing were applied to a dataset consisting in two different quality class Iberian ham samples. Their ability to infer chemical information from samples was tested by comparing the classification results obtained from Partial Least Squares Discriminant Analysis (PLS-DA) and their Variable Importance for Projection (VIP) scores.

Cholinergic control of chaos and evidence sensitivity in a neocortical model of perceptual decision-making

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Abstract. Perceptual decision-making in the brain is commonly modeled as a competition among tuned cortical populations receiving stimulation according to their perceptual evidence. However, the contribution of evidence on the decision-making process changes through time. In this regard, the mechanisms controlling the sensitivity to perceptual evidence remain unknown. Here we explore this issue by using a biologically constrained model of the neocortex performing a dual-choice perceptual discrimination task. We combine mutual and global GABAergic inhibition, which are differentially regulated by acetylcholine (ACh), a neuromodulator linked to enhanced stimulus discriminability. We find that, while mutual inhibition determines the phase-space separation between two stable attractors representing each stimulus, global inhibition controls the formation of a chaotic attractor in-between the two, effectively protecting the weakest stimulus. Hence, under low ACh levels, where global inhibition dominates, the decision-making process is chaotic and less determined by the difference between perceptual evidences. On the contrary, under high ACh levels, where mutual inhibition dominates, the network becomes very sensitive to small differences between stimuli. Our results are in line with the putative role of ACh in enhanced stimulus discriminability and suggest that ACh levels control the sensitivity to sensory inputs by regulating the amount of chaos.

Keywords: Acetylcholine, Cortical model, Decision-making, Chaos.

ACKNOWLEDGMENTS

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Noninvasive Assessment of Inspiratory Muscle Neuromechanical Coupling using Surface Mechanomyography and Electromyography

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The diaphragm is the principal respiratory muscle and accounts for most of inspiratory work. Diaphragm neuromechanical coupling (NMC) is the efficiency of conversion of neural activation to transdiaphragmatic pressure (P_{di}), and is increasingly recognized to be a useful clinical index of diaphragm function and respiratory mechanics in neuromuscular weakness and cardiorespiratory disease. However, the current gold standard assessment of diaphragm NMC requires invasive measurements of P_{di} and crural diaphragm electromyography ($oesEMG_{di}$), which complicates the measurement of diaphragm NMC in clinical practice.

We compared, for the first time, invasive measurements of diaphragm NMC (iNMC) using the relationship between P_{di} and $oesEMG_{di}$, with noninvasive assessment of NMC (nNMC) using surface mechanomyography ($sMMG_{lic}$) and electromyography ($sEMG_{lic}$) of lower intercostal chest wall inspiratory muscles [1]. The study was carried out in collaboration with the respiratory muscle physiology group at King's College London, in the framework of two Long-Term Research Fellowships of the European Respiratory Society (ERS LTRF 2015-5185 and ERS LTRF 2017 01-00086). Both invasive and noninvasive measurements were obtained simultaneously in 12 healthy subjects during an incremental inspiratory threshold loading protocol. Myographic signals were analysed using fixed sample entropy (fSampEn), which is less influenced by cardiac artefacts than conventional root mean square.

We found a curvilinear relationship between invasive P_{di} and $oesEMG_{di}$ measurements (Figure 1a), such that there was a progressive increase in iNMC with increasing inspiratory threshold load (Figure 1d). By contrast, a linear relationship between noninvasive $sMMG_{lic}$ and $sEMG_{lic}$ measurements was observed (Figure 1b and 1c), resulting in little change in nNMC with increasing inspiratory load (Figure 1e and 1f). Progressive recruitment of lower ribcage muscles, serving to enhance the mechanical advantage of the diaphragm, may explain the more linear relationship between $sMMG_{lic}$ and $sEMG_{lic}$ (both representing lower intercostal plus costal diaphragm activity) than between Pdi and crural $oesEMG_{di}$.

Our findings suggest that noninvasive indices of NMC derived from $sEMG_{lic}$ and $sMMG_{lic}$ may prove to be useful indices of lower chest wall inspiratory muscle NMC, particularly in settings that do not have access to invasive measures of diaphragm function.

This work was supported in part by the Generalitat de Catalunya (CERCA Programme and GRC 2017 SGR 01770), the Gobierno de España (RTI2018-098472-B-I00 MCIU/AEI/FEDER, UE), and the Biomedical Research Networking Centre in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN, Instituto de Salud Carlos III/FEDER). M. Lozano-García and L. Estrada-Petrocelli were the recipients of two European Respiratory Society Fellowships (ERS LTRF 2015-5185 and ERS LTRF 2017 01-00086 respectively).

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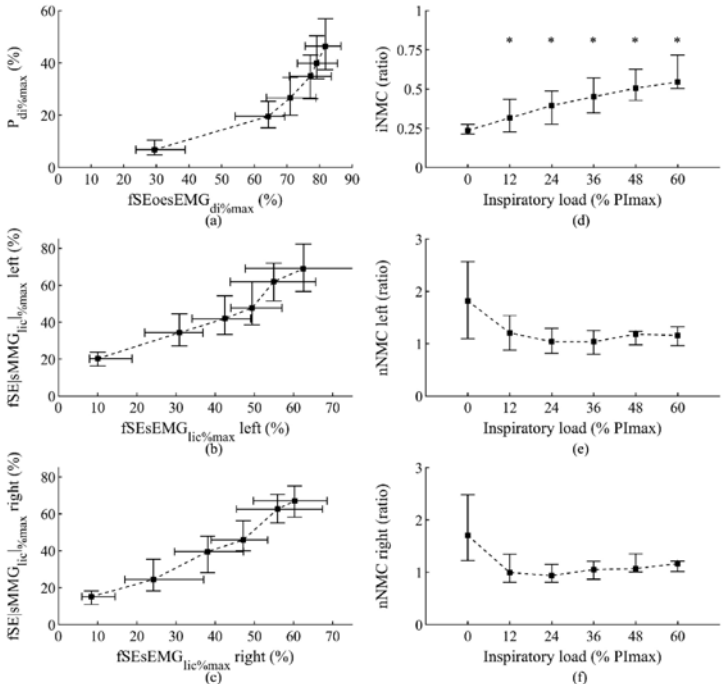

Figure 1. Relationship between electrical and mechanical measures of inspiratory muscle activation, and the corresponding NMC ratios, recorded during the incremental inspiratory threshold loading protocol. Invasive (iNMC) and noninvasive (nNMC) NMCs were calculated as the ratios of Pdi%max to fSEoesEMGdi%max and fSEIsMMGlicl%max to fSEsEMGlic%max, respectively. Data points represent median and interquartile range of the study subjects for each load. All data points with a * symbol were significantly different to each other.

Design of experiment for optimization of volatiles compounds extraction in urine analysis

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The organic compounds that are present in the liquids (LIQs) from the human body such as blood, urine, sweat and saliva; and also in the gases produced (VOCs) such as breath or gas phases of the previously mentioned liquids or stool can give a lot of information about the metabolomics procedures that are occurring in the body. Alterations in the VOCs can give information about any alteration in the normal performance of the body.

Urine is a good sample because is available in large quantities than blood, is obtained in a non- invasively manner, and mostly free from interfering proteins or lipids. Urine is quite chemically complex, because it is not only a biofluid of waste breakdown products of foods and beverages, drugs, and environmental contaminants, endogenous waste metabolites and bacterial by- products (Bouatra et al., 2013); it is also a good clinical chemistry matrix with already identified and accounted up to ~3100 small molecules in human urine (https://urinemetabolome.ca/).

An optimization of the parameters for the urine volatiles was done in order to find the best conditions for the measures. It was created a Design of Experiments (DOE) (2^3) corresponding to 8 experiments (2 levels and 3 variables) and 3 central points (figure 1) to optimize the conditions of sample extraction. The evaluated variables were the quantity of salt added in the vial, the time and temperature of extraction (table 1).

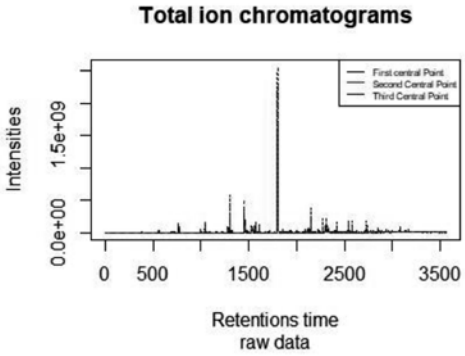

Figure 1. Total Ion Chromatogram (TIC) of 3 of the samples analyzed

Levels	SPME Exposure T[a]	SPME Exposure Time	NaCl
-1	40	20	0.3 gr
+1	60	50	1 gr

Table 1. Evalutaed variable in the DOE with their respective test values
The type of fiber (75 µm-Carboxen/PDMS) (figure 2), the incubation time (20 min) and incubation temperature (40oC) were kept constant. A pool of urines was created and used in all experiments. It was collected the first urine of the day of heath volunteers including man and woman. The pH of the urine pool was adjusted to acid using HCl (5M) and an aliquot of 3 ml was added in each vial of the design. The answer used as the response to evaluate the design was the total area of the chromatograms after baseline correction.

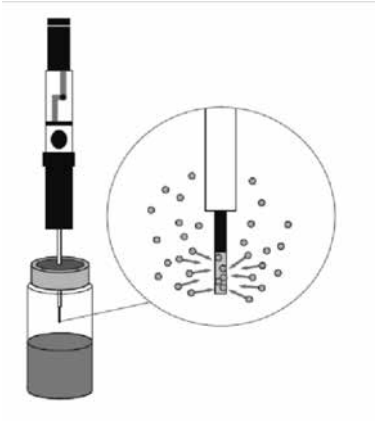

Figure 2: Scheme of the SPME procedure for capturing VOCs

Outstanding sensitivity and selectivity in biomarker detection for medical diagnostics

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Reliable in-time monitoring of diseases is crucial in medical diagnosis and analytics since selecting the most effective therapy is vital for physicians in early stages of disease progression. Thus, being able to selectively detect specific biomarkers for diseases drastically increases the chances of successful treatment.

Label-free optical affinity biosensors represent a potent way of reducing the time of analysis as well as providing outstanding sensitivity and selectivity. However, contrary to labeled biosensors, label-free biosensors cannot directly differentiate between a specific response of the analyte and unspecific adsorption of other components (fouling). The surface of the transducer needs to have the ability to selectively capture the biomarker of interest while preventing fouling of other biomolecules or biological entities that are present in complex biological fluids like saliva, urine, or blood.

We utilize antifouling polymer brushes consisting of different types of monomers that drastically reduce or even completely prevent non-specific adsorption from various bodily fluids including cerebrospinal liquid, saliva, urine, and undiluted blood plasma.[1] The introduction of bioactive motifs that are able to selectively capture the desired analyte while maintaining their resistance to non-specific adsorption is another challenging task. We utilize different techniques to introduce bioactivity to state-of-the-art polymer brushes. The immobilization of bioreceptors directly on polymer brushes was achieved by immobilization of streptavidin and subsequent linking of biotin- functionalized oligonucleotides. These oligonucleotides were then targeted with their complementary oligonucleotides that carried antigens able to determine the presence of antibodies against the Epstein-Barr virus infection. The developed biosensor was able to detect different types of antibodies that indicated different stages of the Epstein-Barr virus infection in clinical blood serum samples.[2] Another immobilization technique involved amine coupling of thrombin aptamers to carboxylic groups of antifouling polymer brushes. These aptamers were able to selectively bind thrombin from 10% whole human blood. The limit of detection of this biosensor is

sufficient for the prediction of a thrombotic event and diagnosis of thrombosis.[3-4] In a similar vein, by optimizing polymer brush composition and architecture we were able to detect Hepatitis B (< 0.002 IU·mL^{-1}) from clinical blood serum samples[5] and food pathogens from milk.[6]

The combined results pave the way to the development of new diagnostic tools for direct and rapid detection of biomarkers leading to meliorated diagnosis that concomitantly improves the personalized therapeutic treatment.

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Signal Processing for Gas Chromatography - Mass Spectrometry: Evaluation of compounds related with exacerbation on Chronic Obstructive Pulmonary Diseases (COPD)

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Breath analysis is a powerful and very promise technique for evaluating respiratory diseases with the advantages of the non-invasive nature, unlimited sample supply and the potential to facilitate early diagnostics. The off-line pipeline normally is done collecting the sample in the hospital using cartridges and/or bags, followed by a preconcentration step, as solid phase micro extraction (SPME), when necessary, and the analysis itself that in the most of cases is done by gas chromatography(GC). Three liters Tedlar® bags were used to collect the total amount of exhaled air by the patients. All bags were cleaned after used flushing with argon and baking at 45 oC (three times).All the samples were collected in the same room and the patients breathe by humidity filter during three minutes before sample collection to the bags. After collection of the samples in the hospital the bags were carried out to the laboratory in ambient temperature and analyzed in the same day of sample collection. The ambient air in each day of collection was also stored in a bag and analyzed using the same procedure as the real samples. For each day of sample collection ambient air, controls and cases samples were collected. For avoiding sex cofounding factor only breath from woman were collected. In the laboratory Solid Phase Micro Extraction (SPME) was used as preconcentrate method. SPME analysis were carried out using a 75 µm carboxen®/ Polydimethylsiloxane (CAR/PDMS) fiber. The obtained gas chromatograms were evaluated on R programming language. The package enviGC were used to visualize the raw total ion chromatograms (TIC). PCA analysis of all samples using the TIC was done to verify the presence of outlier samples. XCMS package was used to extract the features of all chromatograms and matched filter algorithm was used to pick peaking step followed by a group step and alignment. A second group was applied followed by data imputation. The extracted features were then corrected using log transformation and PQN normalization. Machine learning algorithm Partial Least Squares – Discriminant Analysis (PLS-DA) was used to create and evaluate the obtained models. Preliminary results show that breath analysis followed by SPME preconcentration and GC-MS could be used to evaluate patients presenting COPD exacerbations.

Combining HRV and pulmonary function related markers to explain cardiac comorbidities in COPD patients

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Chronic obstructive pulmonary disease (COPD) patients exhibit decreased heart rate variability (HRV), while comorbidities may worsen the patients' prognosis and accelerate disease progression. In this work we have investigated whether HRV analysis, together with clinical markers of disease severity and respiratory function, can be useful to explain the presence of cardiac-related comorbidities in this population. To do this, several HRV indices were evaluated in 46 COPD patients during basal segments before performing a 6-minute walk test (6MWT) [1]. The maximum heart rate (HRmax) and walked distance (Dist) were measured when the test ended, while the heart rate recovery (HRR) marker was estimated immediately afterwards [2]. These features together with the patient characteristics and pulmonary variables were used as input dataset to identify patient with cardiac- related comorbidities (COPDco, n=11). A logistic regression classifier with LASSO regularization was used for modeling and feature selection [3], while model performance was assessed by leave-one-out cross-validation (LOOCV). Only 4 predictive features were needed to accurately identify comorbidities with overall performance metrics of: AUC=84%, sensitivity=77% specificity=83% (Fig. 2). This reduced feature subset included the ratio given by the forced expiratory volume in one second (FEV_1) and the forced vital capacity (FEV_1/FVC), the normalized HRR at minute 3 of recovery ($HRR3_{no}$), the Borg- scale of exertional dyspnea ($BS_{dyspnea}$) and the normalized LF power (LF_{no}). Increased HR recovery after the 6MWT appears to be linked to cardiac comorbidities ($HRR3_{no}$= 23.7 ± 5.9% vs 16.8 ± 7.4%, p=0.005), although its presence has been associated with a better lung function (FEV1/FVC= 61.2 ± 11.6% vs 45.2 ± 12.3%, p=0.001) and lower survival [4]. These features could provide relevant information for early identification of cardiac comorbidities and highlight the importance of considering different factors beyond pulmonary markers for a more complete assessment of COPD patients.

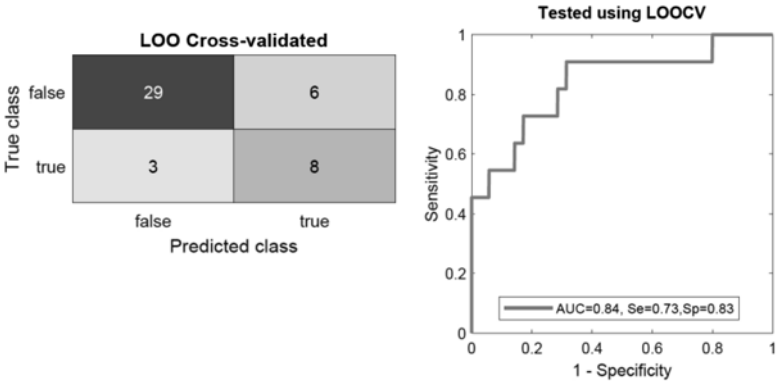

Fig 1. Confusion matrix and ROC curve obtained for the model tested via leave-- one-out cross-validation (LOOCV).

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Mobile wearable biosensors with enzyme-modulated dynamic range for the simultaneous detection of sweat lactate and sweat volume

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We developed a colorimetric analytical platform that simultaneously measures the lactate sweat concentration and the sample volume using a smartphone as a reader. The detection device consists of two contiguous sensors made entirely of paper that can be easily attached to the skin using medical grade tape. The peroxidase-mediated oxidation of 3,3',5,5'-TetramethylBenzidine (TMB) using the H2O2 generated by lactate oxidase (LOX) was used to detect L- Lactate. The dynamic range of the lactate biosensor can be fine-tuned with an enzyme-modulated mechanism. It consists of adding a competitive inhibitor (D-Lactate). This allows to choose between a very low limit of detection (0.06 mM) or a linear response in the physiological range (10-30 mM). The sweat volume sensor contains a gold nanoparticles reservoir. As the wearer sweats, the nanoparticles are carried through the paper strip. This is used to gauge the volume of the sample that has entered in the sensor at a given time by measuring the distance travelled by the nanoparticles with a smartphone. This allows to rectify the colorimetric signal from the lactate biosensors and make it independent from the sample volume. The sweat sensor can be adapted to measure different sample volumes according to the user needs. Using this device we were able to measure lactate levels during an exercise routine in a group of volunteers independently of the wearer's sweat rate. L-Lactate values obtained with the sensor were similar to those obtained with a reference method. Furthermore, the detection platform can be adapted to measure other biomarkers in sweat by changing the enzyme. This, along with the highly customizable design, makes the proposed analytical platform promising for e-health applications.

Nanostructural characterization of influenza virus-like particles with super resolution microscopy

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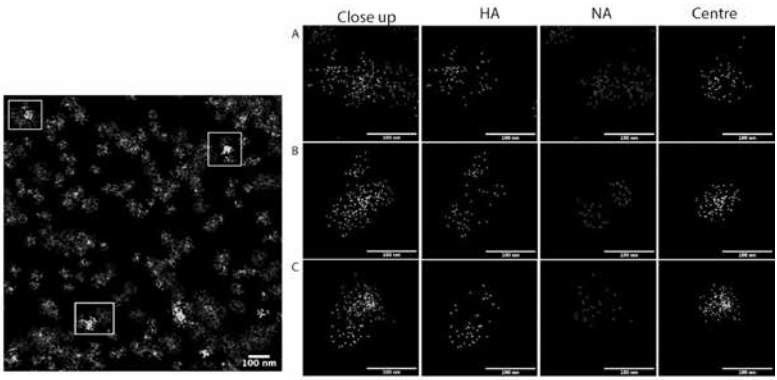
As we unfortunately know, respiratory viruses present risks against human beings, with influenza being one of the most common. The effects of influenza virus in our society are remarkable, being the cause of at least 5 pandemics between the XX and the XXI century, causing more than 60 million deaths[1] and creating the need of seasonal yearly vaccination.

The lack of a universal vaccine boosted the industry to develop novel methods to produce them. Currently, the industry is starting to focus attention on vaccines based on recombinant proteins that can be produced in a safe environment with the DNA of the virus but lacking risks. One type of recombinant vaccine we are focusing on is virus-like particles (VLP), structures that mimic the viral particles without the genetic material, self- assembling and releasing spontaneously from recombinant viral proteins expressed on cells[2]. VLPs contain repetitive, high density and natural motives from the virus showing also promising results as nanovectors since resemble the infectivity machinery of the virus[3]; they are so promising that there are 13 preclinical candidates for COVID-19 vaccine[4].

Despite the interest of VLPs, there is a lack of understanding of how the influenza VLP structure distribute the three main viral envelope proteins along the surface, due to the small size of influenza (100 nm). To overcome this limitation, single molecule localization microscopy (SMLM), a super-resolution technique, has burst in the last years since allows the analysis of the spatial arrangements of molecules in the nanoscale by the localization of fluorophores[5].

Here, we study with SMLM the spatial distribution of each of the three proteins of influenza VLP in the nanoscale. By using DNA-PAINT[6], a SMLM technique that allows us the quantification of target structures, we characterized the differential expression of the three transfected proteins of the VLP on the membrane of mammalian cells. We could identify a heterogeneous expression of the three proteins expressed within the transfected cell population, not being constant for each protein. We also imaged the population of the VLPs produced, measuring semiquantitatively the amount of each protein intra-and inter- particle and characterizing the distribution of them inside the VLP. Further, we could detect a huge heterogeneity of the amount and distribution of proteins; not only they are not evenly distributed but the amount of proteins per VLP is very variable.



To conclude, the heterogeneity detected by SMLM in the protein expression of VLPs could be crucial to understand the hits and miss of VLPs for clinical purposes.

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Heparin Administered to Anopheles in Membrane Feeding Assays Blocks Plasmodium Development in the Mosquito

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Current chemotherapeutic approaches against malaria focus on targeting the asexual blood- stage parasites responsible for the symptoms of the disease. However, the number of parasites in a mosquito is several orders of magnitude lower than in the bloodstream of a malaria patient, which opens potential perspectives for the development of new antimalarial treatments. Specifically, ookinete adhesion to the mosquito midgut is a critical step in the life cycle of the parasite, and therefore a possible target for future transmission-blocking strategies.

Previous studies had demonstrated that the sulfated glycosaminoglycan (GAG) chondroitin sulfate is used as host ligand by the ookinete to cross epithelial cells in the mosquito midgut. Heparin, another sulfated GAG, has a targeting activity towards the ookinete surface. Using membrane feeding assays, we have observed that heparin and hyper-sulfated heparin can block the parasite development in a dose-dependent manner, and we aim to characterize the corresponding mechanism to translate it into a novel transmission-blocking approach.

ACKNOWLEDGMENTS

This work was funded by (i) Spanish Ministry of Science, Innovation and Universities (http://www.ciencia.gob.es/), grant numbers PCIN-2017-100 and RTI2018-094579- B-I00 (which included FEDER funds), (ii) ERA-NET Cofund EURONANOMED (http://euronanomed.net/), grant number 2017-178 (NANOpheles) and (iii) GHTM (UID/Multi/04413/2013). We acknowledge support from the Spanish Ministry of Science, Innovation and Universities through the "Centro de Excelencia Severo Ochoa 2019-2023" Program (CEX2018-000806-S), and support from the Generalitat de Catalunya through the CERCA Program.

Role of Valency on the Ability of ICAM-1-Targeted Nanoparticles to Effectively Cross the Blood-Brain Barrier and Deliver Therapeutic Enzymes in Cellular and Animal Models

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The interaction of drug delivery systems with tissues is key for their application. An example is that of drug carriers targeted to endothelial barriers, which can be transported to intra-endothelial compartments (lysosomes) or transcellularly released at the tissue side (transcytosis). Although carrier targeting valency and affinity influence this process, the mechanism is unknown. We studied this using polymer nanocarriers (NCs) targeted to intercellular adhesion molecule-1 (ICAM-1), an endothelial-surface glycoprotein whose expression is increased in pathologies characterized by inflammation.

METHODS. Polystyrene NCs were used to avoid degradation which may confound trafficking results. NCs were coated with mixtures of anti-ICAM+control IgG at varying molar ratios, to vary targeting valency without modifying other properties. All NCs were ≈180 nm diameter, ≈0.19 PDI, ≈–28 mV, and had ≈8,000 total antibody molecules/μm^2 NC surface, and 7,703, 4,145, and 2,058 anti-ICAM molecules/μm^2 of NC surface (termed high, intermediate, and low valency). Anti-ICAM NCs were labeled with ^{125}I for radiotracing or FITC for microscopy, loaded or not with a therapeutic enzyme, and studied in cell cultures using Transwell filters or *in vivo* after intravenous administration in mice.

RESULTS. A bell-shaped relationship was found between NC targeting valency and transcytosis: high and low NC valencies rendered less efficient transcytosis than an intermediate valency formulation. This is because NC valency played an opposite role in the two sub-processes involved in transcytosis: NC binding-uptake depended directly on valency and exocytosis- detachment was inversely related to this because for a very tight NC-receptor interaction, the post-transport NC-receptor detachment process was compromised. Since transcytosis encompasses both events, the full process finds an optimum at the intersection of these inverted relationships, explaining the bell-shaped behavior. NCs trafficked to lysosomes from the apical side

and from the basolateral side for high valency NCs which are slower at detaching from the receptor, resulting in an inverted bell-shape relationship between NC valency and lysosomal trafficking. Anti-ICAM NCs trafficked into the brain after intravenous injection in mice, and both cellular and *in vivo* data showed that intermediate valency NCs resulted in higher delivery of therapeutic acid sphingomyelinase, required for types A and B Niemann-Pick disease.

CONCLUSIONS. A balance must be achieved where NCs have enough targeting valency for apical binding and uptake without hindering basolateral detachment or promoting massive lysosomal trafficking. This finding offers critical insight into the parameters ruling transcytosis of receptor- targeted NCs and the application of this knowledge to optimize therapeutic delivery across endothelial linings.

Personalizing pediatric cancer treatment with dynamic BH3 profiling.

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Pediatric cancers represent less than 1% of all newly diagnosed cancers each year, yet they are the second leading cause of death in children, and chemotherapy used to treat them induce devastating secondary effects. In this work we focus our efforts on finding new therapies with less undesired toxicities to treat two main pediatric cancers: rhabdomyosarcoma (RMS) and B- cell acute lymphoblastic leukemia (B-ALL). Our aim is to personalize the treatment of refractory/relapsed patients.

When exposed to the right therapy cancer cells die by apoptosis. This type of cell death is regulated by the BCL-2 family of proteins comprising effectors (BAX and BAK), activators (BIM, PUMA and BID), sensitizers (BAD, NOXA, HRK...) and antiapoptotic proteins (BCL-2, BCL-xL, MCL-1...). In response to anticancer treatments, cancer cells often present antiapoptotic adaptations to survive therapy. To overcome this resistance, BH3 mimetics -small molecules that inhibit antiapoptotic proteins- are being developed to block resistance of cancer cells (like venetoclax for BCL-2, and others currently explored in clinical trials). The key question is when and how to use these new promising inhibitors.

In this regard, dynamic BH3 profiling (DBP), a functional predictive assay that measures net changes in mitochondrial apoptotic signaling (Δ% priming), anticipating cell death days/weeks in advance, has been successfully validated *in vitro*, *in vivo* and on patient samples. Furthermore, DBP can also detect antiapoptotic adaptations upon treatment, guiding the use of BH3 mimetics to overcome therapy-induced resistance.

We used DBP to test new therapeutic strategies for RMS and B-ALL. By performing DBP we were able to identify which treatments caused an increased apoptotic priming and which antiapoptotic adaptations were induced after treatment. We confirmed our predictions by analyzing cell death at longer timepoints, observing a good correlation with our Δ% priming predictions. In addition, based on the antiapoptotic adaptations observed using DBP, we identified effective synergistic combinations of

several anticancer therapies with BH3 mimetics both in B-ALL and RMS cell lines. Specifically, in RMS, we used a combination of the common chemotherapeutic drug vincristine with a selective MCL-1 inhibitor in a patient-derived xenograft (PDX) mouse model, causing a significant tumor shrinkage when combined, confirming the predictive capacity of DBP.

Finally, we used our assay to predict response to treatments in relapsed/refractory pediatric patients and help clinicians to choose the best therapy for these complicated cases.

A clearing protocol for Galleria mellonella larvae: Visualization of internalized fluorescent nanoparticles

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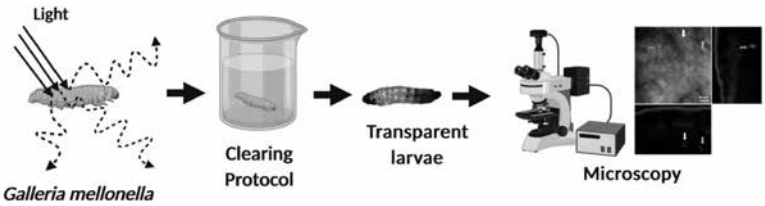
Light scattering is a challenge for imaging three-dimensional organisms. A number of new tissue clearing methodologies have been described in recent years, increasing the utilities of clearing techniques to obtain transparent samples. Here, we describe the optimization of a suitable and novel protocol for clearing Galleria mellonella larvae, an alternative infection animal model with a promising potential for the toxicological evaluation of different molecules and materials. This has allowed the visualization of internalised fluorescent nanoparticles using confocal microscopy, opening the door to a wide range of different applications.



In Vivo Photocontrol of Adrenergic Neurotransmission

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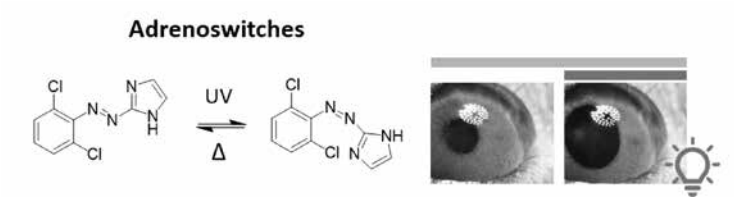
Adrenoceptors are ubiquitous and regulate most vital functions in the human body, including heart and respiratory rate, digestion, smooth-muscle contraction, gland secretion, and pupil diameter among others. In addition, adrenergic neurons firing from the locus coeruleus towards different areas of the central nervous system mediate alertness, responses to acute stress and danger, pain modulation, arousal, sleep-wake cycles, as well as neuroplasticity and cognitive behaviour. Despite the physiological relevance of adrenergic neurotransmission, molecular methods to precisely modulate the activity of endogenous adrenoceptor and to functionally dissect their pathways *in vivo* are not available.

Here we present a set of photochromic ligands, that we call adrenoswitches, to switch on and off adrenoceptor activity with high spatio-temporal resolution. Using a non-canonical azologization approach, we have designed novel arylazoheteroarene units that we have characterized *in vitro* and in two animal models (zebrafish locomotion and pupillary reflex in mice). The drug-like properties of these molecules, their efficacy and absence of acute toxicity in zebrafish larvae, and most remarkably the fact that specific adrenergic photomodulation was readily and reversibly achieved in the mammalian eye by topical application without formulation, all indicate that adrenoswitches could be a disruptive tool to dissect physiological adrenergic signaling and to develop safe and effective therapies. For example, photocontrol of adrenoceptors at specific locations might allow to single out individual adrenergic projections from the locus coeruleus, or to selectively decouple pupil tone from environmental illumination.



ACKNOWLEDGMENTS

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Using genomics to map amyloid nucleation in Alzheimer's disease

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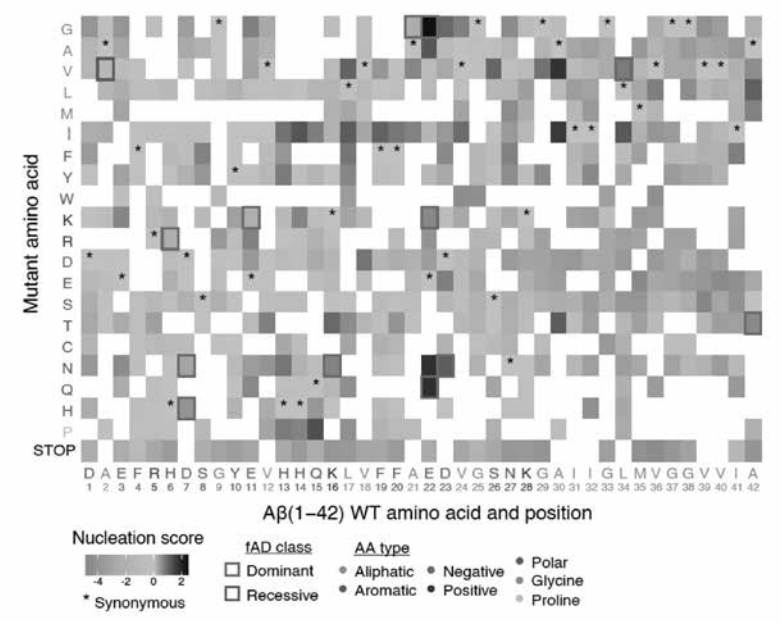
Alzheimer's Disease (AD) is the most common form of dementia and a leading global cause of human mortality and morbidity. There are no effective preventions or cures for Alzheimer's despite decades of research, suggesting a fundamental lack of understanding of the causes of the disease.

Amyloid plaques of the amyloid beta (Aß) peptide are a universal pathological hallmark of AD and mutations in Aß cause familial forms of AD (fAD). Moreover, these known disease-causing mutations are only a tiny fraction of the possible mutations in Aß. The vast majority of mutations in Aß are of completely unknown clinical significance and yet, given the human population size and mutation rate, each of these mutations is likely to exist in one or more people currently alive on the planet.

We used deep mutagenesis to quantify *in vivo* amyloid fibril nucleation for >14,000 variants of Aß. This provides the first comprehensive description of how mutations alter the nucleation of any amyloid fibril. Our results reveal a modular organisation 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 the nucleation of amyloid fibrils. Strikingly, the *in vivo* nucleation scores, unlike computational predictors and previous measurements, accurately discriminate all the known dominant 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 genetic variation in Aß, opening a new path for personalized therapy in AD.



Heatmap of nucleation scores for single aa mutants. The WT aa and position are indicated in the x-axis and the mutant aa is indicated on the y-axis, both coloured by aa class. Variants not present in the library are represented in white. Synonymous mutants are indicated with '*' and fAD mutants with a box, coloured by fAD class.

Nanoscale Mapping of the Conductivity and Interfacial Capacitance of an Electrolyte Gated Organic Field Effect Transistor under Operation

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Biological systems and materials interact via complex mechanisms spanning different temporal and spatial scales. Probing nanoscale electrical properties at the interface with an electrolyte solution, under externally applied voltages, is of immense importance in the field of organic bioelectronics [1]. The Electrolyte-Gated Organic Field-Effect Transistors (EGOFETs) are one of the widely studied devices for such applications. Here, we present [2] the nanoscale mapping of conductivity and interfacial capacitance of the active channel of an EGOFET under operation using in-Liquid Scanning Dielectric Microscopy [3][4] in force detection mode, as shown in figure below. The local electrostatic force vs gate voltage transfer characteristics correlates well with the global current-voltage transfer characteristics, offering the possibility of optimisation and control. The nanoscale mapping captured minute electrical heterogeneities corresponding to the variation in interfacial capacitance resulting from the presence of ultrathin non-uniform insulating layer in the vertical phase-separated organic semiconducting blend.

The present work elucidates the nanoscale properties at the scale down to ~100nm, thereby offers the possibility to study transduction mechanism happening at the organic semiconductor/electrolyte and cell/electrolyte interfaces. It holds the potential to act as a tool for substantial optimisation of organic electronic devices for bioelectronic applications such as electrical recording of excitable cells or label-free biosensing.

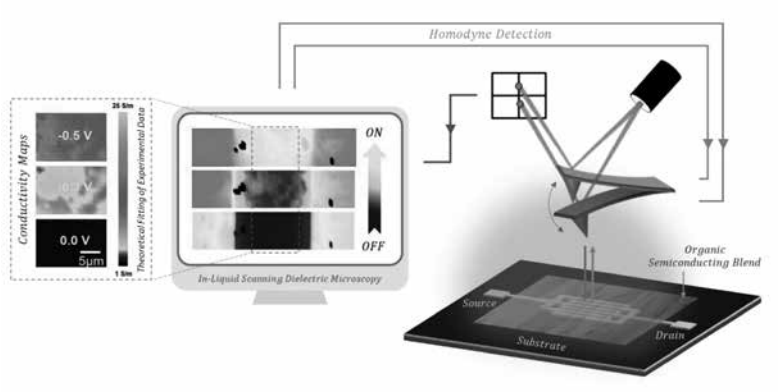

Figure (a) In-liquid SDM setup for the nanoscale electrical characterization of a functional EGOFET with conductive cantilever as both gate electrode and force sensor; Conductivity maps at different gate voltage obtained by theoretically fitting each electrical curve at each pixel.

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Mobile Paper Biosensors for Screening Elevated Cytokine Levels in Blood Samples from COVID-19 Patients

Cristina Adrover Jaume

The SARS-CoV-2 pandemic has rapidly spread worldwide with enormous social and economic repercussions. During the pandemic peak, saturation of hospitals forced healthcare providers to decentralize COVID-19 care. For this reason, there is an urgent need for home testing biomarkers that indicate progression to severe COVID-19.

In order to cover this need, we have designed a rapid test for detecting prognostic biomarkers of COVID-19 in a small drop of blood. It consists of an immunosensor made solely of paper that only requires a smartphone to read colorimetric signals. The immunosensors have a 3D design that allows the vertical transfer of antibody-decorated nanoparticles from a reservoir to capture areas. There, antibody-antigen interactions result in the formation of colored spots in the paper substrate that are quantified in seconds with a mobile app, and the color intensity of the signals depends on the number of antibody-antigen interactions.

These immunosensors were utilized for detecting IL-6. Cytokines have been identified as key players in a hyperinflammatory syndrome or “cytokine storm” responsible for many severe cases of COVID-19. However, cytokines are found at low basal levels (typically below 5 pg mL^{-1}), which makes it challenging to detect them in blood samples. Here we show that our biosensors can detect IL-6 with a limit of detection (LOD) of only 0.01 pg mL^{-1}. With this low LOD it was possible to dilute blood samples in order to detect IL-6 in the physiological range without the need to separate cells from plasma or perform any additional purification step. This method allowed us to differentiate samples from healthy donors from samples with high levels of IL-6 obtained from COVID-19 patients.

Identification of new collagen-associated biomarkers in lung cancer by advanced image analysis of patient biopsies

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[*]*Both authors played an essential role in the project.*

Lung cancer is the most common cause of cancer-related deaths. The two most common lung cancer subtypes are adenocarcinoma (ADC) and squamous cell carcinoma (SCC). Tumor-associated fibroblasts (TAF) are essential regulators of tumor progression and are the main responsible for the abnormal fibrillar collagen (types I and III) deposition within the tumor stroma, which is associated with increased stiffness and adverse outcome. Although the quantity of collagen fibers has been analyzed in many cancer types including lung cancer, the structure and topography of collagen fibers remains undetermined. We hypothesize that collagen architecture may include new biomarkers that may improve current diagnosis and/or prognosis of lung cancer patients. To test this hypothesis, we have analysed the architecture of collagenous fibers in histological sections of ADC and SCC patients, stained with picrosirius red, which highlights fibrillar collagens selectively. Polarized microscopy was employed to exploit the refractive properties of the dye and image collagen fibers, whose morphology and arrangement were measured with an open-source fiber recognition software, CT-FIRE, which provides quantitative features describing the single fibers (Straightness, Length, Width) and their arrangement (Alignment, Number of Fibrils) in the image. Artificial images were initially employed to test the quality of the recognition and the optimal parameters were chosen for the analysis on patients' samples. Finally, the fibrillar features extracted from ADC and SCC images were compared, showing differences in fibers density, arrangement and morphology. This work in progress confirms the evidence of a strong fibrotic response in ADC compared to SCC. Furthermore, it paves the way for a more complete characterization of the different fibrotic response in patients' stroma between ADC and SCC subtypes, which might improve patients' prognostic stratification.

Correlating super-resolution microscopy and electron microscopy to study size and ligand heterogeneity in nanoparticles

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The use of nanoparticles (NP) for the delivery of therapeutics (nanomedicine) is a promising research area, with a myriad of different formulations being developed. A common characteristic is the decoration of the NP with ligands to target biomarkers of interest. However, heterogeneity found in size and ligand distribution can alter formulation performance. Yet, these parameters - and more importantly the relationship between them - are seldom investigated, due to a lack of standardized methods. Correlating super-resolution microscopy (SRM) with electron microscopy (EM) offers a powerful approach to explore unprecedented material characteristics at the nanoscale and at single-particle level. Specifically, SRM can quantify the number of accessible ligands with a resolution down to 20 nm, whilst Transmission EM (TEM) is the method of choice to study size and shape heterogeneity. Despite the potential of this correlative technique, remarkably it has not yet been explored in the field of nanomedicine. Since these parameters highly affect nanoparticle performance, it is necessary to apply a correlative approach to understand their relationship and in turn to optimize formulation design. Polymeric PLGA-PEG-Maleimide nanoparticles are manually formulated via the nanoprecipitation method, with long/short PEG chains and varying maleimide content. The maleimide groups are then conjugated to thiol-DNA oligonucleotides, which behave as a model ligand. Conjugated nanoparticles are imaged using the DNA Points Accumulation for Imaging in Nanoscale Topography (DNA-PAINT) technique. The same region of interest is then imaged in TEM, followed by manual correlation. An overview of the method is found in Figure 1.

Using DNA-PAINT we were able to quantify accessible ligand groups on a variety of PLGA-PEG- maleimide nanoparticles and demonstrate how PEG architecture can influence conjugation efficiency and ligand accessibility, allowing the optimization of the formulation. Using the correlative approach, we showed the relationship between accessible ligands/ligand density and nanoparticle size at different maleimide concentrations and compared the results to theoretical trends.

The correlation of SRM and TEM offers a multidimensional insight into the relationship between nanoparticle size and ligand heterogeneity and could be expanded to other formulations and parameters. This in turn will bring us a step closer to the optimization of successful nanoparticle formulations.

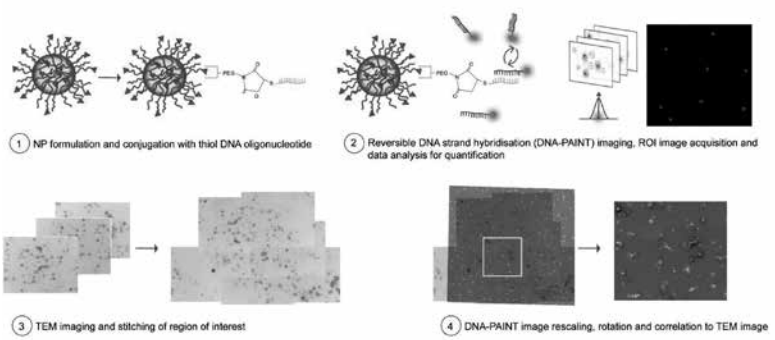

Figure 1. Overview of the super-resolution microscopy and electron microscopy correlation method.

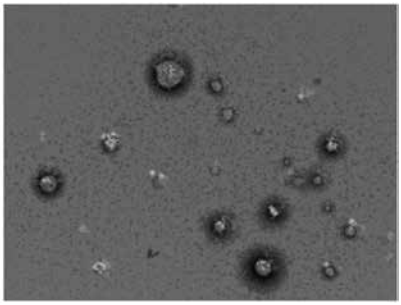

Figure 2. Super-resolution microscopy and transmission electron microscopy correlated example of PLGA-PEG-maleimide nanoparticles.

Design of multivalent polymersomes for range-selective binding

Azzurra Apriceno[1,2], Meng Liu[3,4], Miguel Sipin[2], Edoardo Scarpa[2], Laura Rodriguez-Arco[2], Alessandro Poma[5], Stefano Angioletti-Uberti[4,6], Giuseppe Battaglia[1,2,7,8]

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The success of nanomedicine is nowadays strictly associated to the ability of selectively targeting the site of interest: the more specific a therapy is, the more likely it is to be efficient.

The use of multiple ligands, i.e. multivalency, with low affinity for their receptors has proved to successfully address this 'selectivity' requirement.[1] In fact, thanks to the ligand- receptor simultaneous interactions, the collective binding (avidity) compensates for the low affinity and generates a switch-like behaviour where the binding occurs only above a certain receptors density.[2]

This mechanism, widely spread in nature for the mediation of several aspects in cell biology[3] [4]. has recently inspired the design of several drug delivery polymeric nano-constructs functionalized with suitable ligands to guide the carriers according to the level of receptors expression and to deliver their cargos where most needed.[5]

Polymersomes, self-assembled amphiphilic diblock copolymer vesicles, have been widely used as nano tools for drug delivery purposes thanks to their versatility and increased stability within biological environments. A recent research carried out on pegylated polymersomes has shown, through cellular uptake studies, that these nanovesicles can display a superselective behaviour by tuning the effective contribution, in terms of chemical potential, of different parameters including the polymer brush length, particle sizes, ligands number.[6]

Taking inspiration from these results, the main purpose of the present study was to explore a new type of selectivity where multivalent polymersomes only bind targets when the receptor density is within a certain range. To this aim, a statistical

mechanical modelling study was firstly carried out in order to characterise the region where to expect the range selective targetin. Then, Angiopep2-decorated polymersomes were prepared by a solvent switch approach. The formulations were characterised by DLS and TEM and binding studies between functionalized polymersomes and highly-expressing LRP1 FaDu cell line were carried out through fluorometer and laser confocal scanner microscopy.

Understanding the behaviour of the modelled system will significantly improve the design of precise nano-devices with the ability of performing ad hoc selective transport and site targeting within cellular environment.

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An injectable nanobiosensor for continuous remote monitoring of cancer patients

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Remote patient monitoring (RPM) of cancer diseases can be potentially used to increase current predictive rates, while contributing for a more cost-effective and accessible diagnosis and treatment. Patients at high risk of cancer recurrence would constitute an ideal population for such improved cancer monitoring tools. These novel tools should have the ability to remotely monitor patient data, which should be used to detect disease onset or progression [1].

Herein, we propose the development of remote monitoring tools based on biomaterials and nanotechnology for high-risk profile cancer patients. The main objective is to develop a minimally invasive and biocompatible implantable biosensor to be used in the early tumour surveillance in post-operative prostate cancer patients. For this, a hydrogel was combined with plasmonic nanoparticles for cancer monitoring by means of surface-enhanced Raman scattering (SERS) spectroscopy. SERS is a very powerful analytical technique with many applications in the medical field. [2] The main function of SERS, will be to detect the cancer biomarkers that will be close to the nanoparticles. In this specific case, gold nanostars (GNSs) [3][4], were selected as the plasmonic material to embed in the hydrogels. Different size ranges and concentrations of GNSs and their corresponding optical properties were tested before their combination with the hydrogels. After TEM, UV-vis and SERS characterisation using a well-known Raman reporter (MB: methylene blue) the nanoparticles were combined with hydrogels at different concentrations. After that, the GNSs loaded hydrogels were analysed by SERS spectroscopy. The results show that the hydrogels with GNSs concentration of 5 mM have a higher signal than those having 1 mM concentration of gold, as expected. The homogeneity of the final biomaterial was tested by performing intensity Raman maps of an area of 100 × 100 µm for the hydrogels without GNS, with GNSs and with GNSs labelled with the Raman reporter. The high homogeneity and reproducibility of these substrates make them ideal candidates for their use in the monitoring of biomarkers in body fluids through an intradermal implant.

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Improving self-propulsion of urease-powered micromotors

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Enzyme coated micro- and nanomotors self-propel by the biocatalytic conversion of substrates into products and show great promise as active drug delivery cargos.[1] However, many of the fundamental aspects underlying enzyme-powered self-propulsion have yet to be understood, and are crucial for their biomedical implementation.[2] For this reason, we aim to improve the self- propulsion of urease-powered micromotors (UR-motor) by studying the influence that both a) enzyme purity and b) ionic species in the media have on the self-propulsion performance.

Commercial preparations of enzyme contain protein contaminants, hence, we investigated how enzyme purity affects its loading efficiency onto the microparticles. Urease purification improves the speed of the micromotors 3-fold when compared to micromotors functionalized with unpurified.

Ionic species are ubiquitous in biological fluids, therefore we also studied the effects of ionic species on the self-propulsion of UR-motor.[3] Results showed that the presence of PBS, NaOH, NaCl and HEPES reduced self-propulsion of UR-motors. In order to mitigate the effect of ionic species on micromotors' performance, we coated the motors with methoxypolyethylene glycol amine (mPEG) showing higher speed compared to non-coated motors at intermediate ionic concentrations.

This work represents a significant improvement to the UR-motor performance by increasing enzyme loading through the elimination of undesired proteins. Moreover, we made enzymatic micromotors more tolerant to ionic species found in biological environments. Altogether, these results provide new insights into the feasibility of implementation of urease-powered micromotors and the understanding of its motion mechanism.

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The ESCRT-III machinery from Plasmodium falciparum participates in the biogenesis of extracellular vesicles during infection

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Infection with Plasmodium falciparum enhances the production of extracellular vesicles (EVs) in parasitized red blood cells (RBCs) [1]. In higher eukaryotes, EV biogenesis relies on the action of the Endosomal Sorting Complex Required for Transport (ESCRT) machinery [2]. The core of the ESCRT machinery comprises ESCRT-I, -II, -III, ALIX/Bro1 and VPS4 sub-complexes [2]. Among them, ESCRT-III is highly conserved across the eukaryotic lineage [3]. In our study, two different *P. falciparum* homologues of Vps32 (PfVps32A and PfVps32B), the most abundant protein of the ESCRT-III sub-complex, were identified. Moreover, as the parasite lacks the upstream ESCRT complexes, the presence of a putative PfBro1 protein (whose counterpart in humans can directly recruit and activate ESCRT-III members [4]) was studied. A combination of Western blot and immunofluorescence assays revealed that all the studied proteins are expressed throughout the intraerythrocytic cycle of *P. falciparum*. Furthermore, their presence in purified EVs from a P. falciparum-infected RBC culture was detected by super-resolution microscopy thus confirming their participation in EV formation. Additionally, the action of PfBro1 and PfVps32 was reconstituted using giant unilamellar vesicles as a model system. Our experiments provide evidence that PfBro1 recruits and activates PfVps32 homologues. In turn, PfVps32 triggers bud generation visible by confocal microscopy. Finally, we have studied the potential of these proteins as future vaccine antigens. Growth inhibition assays showed that in *vitro P. falciparum* growth was reduced >50% by polyclonal IgG antibodies raised against PfVps32A, indicating its potential as a therapeutic target.

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On the Spatial Resolution of Nanotomography Based on Scanning Dielectric Microscopy: a Numerical Analysis

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The interest in nanotomographic techniques has piqued over the recent years particularly in the fields of medicine, life sciences and material science. Existing methods for these sub-surface investigations like Scanning Electron Microscope, Transmission Electron Microscopy and X-ray microscopy techniques have their own limitations when imaging soft samples, matrices and living cells which in turn plays a major role in biological sciences. The need for nuanced nanotomographic techniques has shone attention on the Scanning Probe Microscopy (SPM). Among them, Electrostatic Force Microscopy (EFM) 1,2 has been a prime contender owing to its wide application range and efficiency 3,4,5,6. In this present effort, we focus on the theoretical study of the spatial resolution that can be achieved with this technique in nanotomographic investigations with two major objectives. First, to analyze the capabilities of EFM for measuring the lateral distance between individual buried entities. And, second, to identify the limits of EFM to identify objects superimposed in the vertical direction. We show using numerical calculations that EFM can provide a very good spatial resolution on nanoobjects buried below the sample surface under specific conditions and discuss its practical implementation. Our results show that EFM is a good candidate as a nondestructive SPM spatial-resolution technique at subsurface level for applications in material and life sciences.

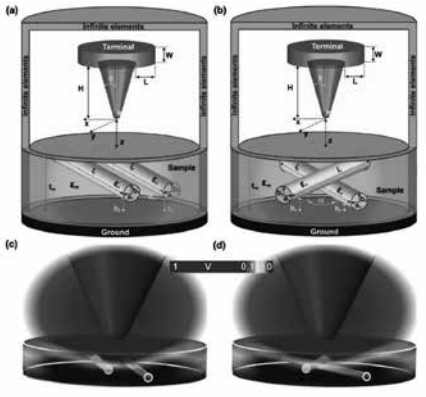

Figure 1. (a)-(b) Schematics of the two EFM systems for buried (a) parallel nanowires and (b) crossed nanowires. (c)-(d) Electric potential distributions corresponding to buried (c) parallel nanowires and (d) crossed nanowires. Parameters used in (c)-(d): tip radius R=100 nm, cone height H=12.5 μm with half cone angle Θ=25º, located at a distance z=20 nm from the sample with thickness t_m=200 nm, dielectric constant ε_m=4 and buried wires of lengths l_1=l_2= 1 μm, semi-axes a_1=a_2=b_1=b_2=25 nm, dielectric constants ε_1=10^3 and ε_2=2, ground-wire distances h_1=70 nm h_2=20 nm, inter-wire distance d_w=200 nm (c) and angle α= 45º (d).

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

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Plasmodium falciparum, the causative agent of the most severe form of malaria, has an overall AT content in its genome of 80.6%, an enrichment only comparable to the amoeba Dictyostellium discoideum [1]. These AT-rich codons tend to encode asparagine residues in *P. falciparum*, which form long low complexity regions (LCRs). Proteins with large LCRs enriched in asparagine and glutamine (N/Q) repeats have a propensity to form insoluble intracellular aggregates [2]. An in depth in silico analysis of the *P. falciparum* proteome found 1,300 proteins with one or more N/Q-rich regions, of which 503 proteins contained domains capable of nucleating aggregation events [3]. Moreover, aggregation in *P. falciparum* live cultures has been detected using an amyloid-specific staining [4].

Unregulated aggregation of proteins has been associated with neurodegenerative diseases and type II diabetes, among other pathologies. However, protein aggregates have also been shown to have functional roles for the cell, e.g. being important in the antiviral innate immunity or in the persistence of synaptic facilitation in mammals. Conformational disorders usually occur when the load of protein aggregates surpasses the handling capacity of the cellular protein quality control machinery. *P. falciparum* is not expected to be an exception to this rule and actually when the chaperone PfHsp110c was knocked down in parasites, they were unable to prevent aggregation of LCR-containing proteins, leading to the pathogen's death [5]. Conversely, inhibiting functional aggregation can also be harmful for some cells, for instance, avoiding amyloid formation of the mammalian protein Pmel17 in melanocytes leads to a leakage of toxic intermediates of melanin and to a reduced melanocyte viability [6].

P. falciparum protein aggregation features could be useful in the design of radically new antimalarial strategies based on the potential toxicity for the parasite of (i) an externally induced aggregation of its own proteome, or (ii) the prevention of protein aggregation, if this phenomenon has a functional role for the pathogen's survival. To explore the first hypothesis, we selected a set of aggregative peptides naturally present in the *P. falciparum* proteome and delivered them to the parasite using cell penetrating peptides and ghost erythrocytes as nanocarriers. To assess if preventing intrinsic protein aggregation is toxic for the parasite, we checked the antimalarial

activity of amyloid pan-inhibitors, a group of compounds that avoid aggregation of amyloid-prone proteins [7], as well as the effect of these molecules on the overall aggregation state of *P. falciparum* by analyzing the ubiquitin expression of parasites after their treatment with amyloid pan-inhibitors.

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Photoswitchable dynasore analogs to control endocytosis with light

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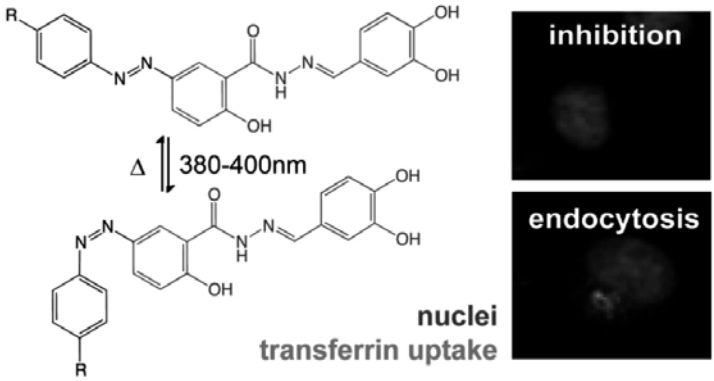
The spatiotemporal control of cellular dynamic processes has great fundamental interest but lacks versatile molecular tools. Dynamin is a key protein in endocytosis and an appealing target to manipulate cell trafficking using patterns of light. We have developed the first photoswitchable small-molecule inhibitors of endocytosis (dynazos), by a stepwise design of the photochromic and pharmacological properties of dynasore, a dynamin inhibitor. We have characterized their photochromism with UV-visible and transient absorption spectroscopy and their biological activity using fluorescence microscopies and flow cytometry. Dynazos are water-soluble, cell permeable, and photostable, and enable fast, single-wavelength photoswitchable inhibition of clathrin- mediated endocytosis at micromolar concentration.



Differential adaptability between reference strains and clinical isolates of Pseudomonas aeruginosa into the lung epithelium intracellular lifestyle

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Intracellular invasion is an advantageous mechanism used by pathogens to evade host defense and antimicrobial therapy. In patients, the intracellular microbial lifestyle can lead to infection persistence and recurrence, thus worsening outcomes. Lung infections caused by Pseudomonas aeruginosa, especially in cystic fibrosis (CF) patients, are often aggravated by intracellular invasion and persistence of the pathogen. Proliferation of the infections species relies on a continuous deoxyribonucleotide (dNTP) supply, for which the ribonucleotide reductase enzyme (RNR) is the unique provider. The large genome plasticity of P. aeruginosa and its ability to rapidly adapt to different environments are challenges for studying the pathophysiology associated with this type of infection.

Using different reference strains and clinical isolates of P. aeruginosa independently combined with alveolar (A549) and bronchial (16HBE14o- and CF-CFBE41o-) epithelial cells, we analyzed host-pathogen interactions and intracellular bacterial persistence with the aim of determining a cell type-directed infection promoted by the P. aeruginosa strains. The oscillations in cellular toxicity and oxygen consumption promoted by the intracellular persistence of the strains were also analyzed among the different infectious lung models. Significantly, we identified class II RNR as the enzyme that supplies dNTPs to intracellular P. aeruginosa. This discovery could contribute to the development of RNR-targeted strategies against the chronicity occurring in this type of lung infection.

Overall our study demonstrates that the choice of the bacterial strain is critical to properly study the type of infectious process with relevant translational outcomes.

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Polymersomes eradicating Tuberculosis

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Tuberculosis (TB) is listed among the top 10 causes of death worldwide, with around 1.8 million deaths per year and affecting one third of the world population, especially in the developing countries.

The etiological agent of TB is the *Mycobacterium tuberculosis (Mtb)*, which attacks mostly the lung macrophages. *Mtb* evolved molecular determinants to prevent phagosomes maturation and it is able to survive within macrophages' phagosomes, thus becoming a challenging intracellular pathogen to eradicate.

Treatments against TB consist of long-term administrations of combined antibiotics (six to nine months), which have the dramatic side-effect of promoting multi-drug resistance. A way to reduce the length of the therapy would be improve drugs efficacy, for example by targeting the pathogen more precisely right within the macrophages' subcellular compartments where it hides.

We developed here a drug delivery system, based on polymersomes, able to release its payload only within the cell cytosol and to thus chase the pathogen. Polymersomes are artificial synthetic vesicles, made of amphiphilic polymers that enclose a water solution, and they can be loaded with molecules such as drugs, peptides or proteins, and nucleic acids' fragments. According to their formulation, polymersomes can be engineered to specifically deliver their cargo (including antibiotics) even to intracellular locations. We synthetize pH-sensitive co-polymers PMPC-PDPA (*poly(2 (methacryloyloxy)ethylphosphorylcholine)-co-poly(2-(diisopropylamino)ethyl methacrylate*) polymersomes, loaded with antimycobacterial drugs, able to reach infected macrophages and release the loaded antimicrobials directly where the *Mycobacteria* reside.

Specifically, we demonstrate polymersomes' biocompatibility, and describe their uptake from macrophages *in vitro*, using monocytes-derived macrophages. Then, we test their efficacy *in vivo* in Danio rerio (zebrafish) infected with *Mycobacterium marinum* (the causative agent of TB in zebrafish and close relative of *Mycobacterium tuberculosis*) that had developed granuloma (a hallmark of TB).

Taken together, our results show the capacity of PMPC-PDPA polymersomes to effectively increase the efficacy of antimycobacterial drugs, being able to significantly decrease, and in some cases eradicate, intracellular infection.

We believe that this method could be employed to improve the current therapy for TB, by reducing the effective dose required, and, at the same time, offering a potential solution to multidrug resistance.

Lamellarity and Electrical Properties of Single Liposomes Measured by In-Liquid Scanning Dielectric Microscopy

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Liposomes are widely used as drug delivery nanocarrier [1, 2, 3] and as cell membrane model systems [4, 5]. Lamellarity, i.e. the number of concentric lipid bilayers, is a key property of liposomes. It determines the stability of liposome preparations [6], the amount of lipophilic drugs that can be encapsulated and the kinetics of its release[3]. Moreover, it also determines the mechanical [7] and dielectric [8] properties of liposomes, which are relevant in their interaction with cells [9, 10] or in its manipulation [11].

Lamellarity has been successfully determined by several single liposome microscopic techniques (cryo- Electron Microscopy, Freeze-fracture Electron Microscopy or Light Microscopy [14, 15] and, more recently, Force-Spectroscopy measurements with the Atomic Force Microscope [7]) which however present some drawbacks (e.g. they are destructive or invasive or require of exogenous labels or staining agents). To overcome these limitations, sub-micrometric non-invasive label-free microscopic techniques to determine lamellarity would be desirable. In the present work, we measure the electric properties of individual liposomes adsorbed on metal electrodes by in-liquid Scanning Dielectric Microscopy in force detection mode. We show that the equivalent homogeneous dielectric constant of the liposomes can be related to the number of lamellae and to the separation between them. Moreover, we are able to provide an accurate determination of the lipid bilayer specific capacitance. The proposed approach offers a unique label-free and non-invasive method to determine the structural and electrical properties of small-scale liposomes and of nanovesicles in general.

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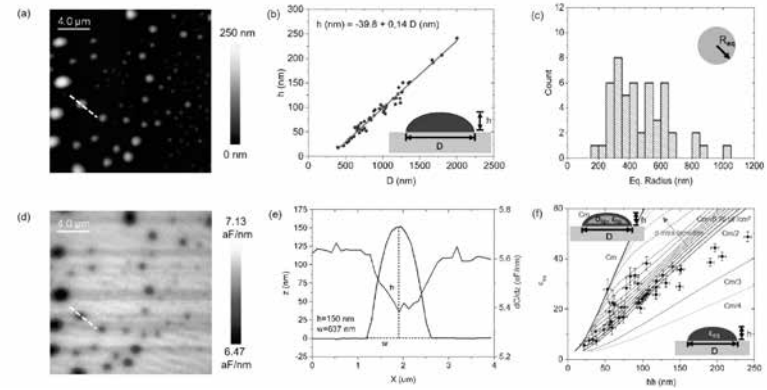

Figure 1.(a) AFM topographic image of DOPC liposomes adsorbed on a functionalized planar gold substrate. (b) Height versus width of the liposomes in (a) (symbols), together with a linear fit to the data (blue line). (c) Distribution of equivalent spherical radii of the liposomes in (a). (d) Constant height SDM image acquired at a distance z=250 nm and at a frequency fel=5 MHz. (e) Cross-section topographic (black line) and capacitance gradient (blue line) profiles along the dashed line in (a) and (d). (f) Lamellarity distribution for all the electrically detected liposomes (d), extracted from the signal at four measuring distances. Experimental parameters: σ_{sol}=0.2 uF/m, σ_{lip}=0.8 S/m, f_{mod}=6 kHz, v_{ac}=0.7 V, k~0.43 N/m, f_0=30 kHz (air).

Mechanical and structural characterization of Quatsome vesicles by AFM and X-Ray techniques.

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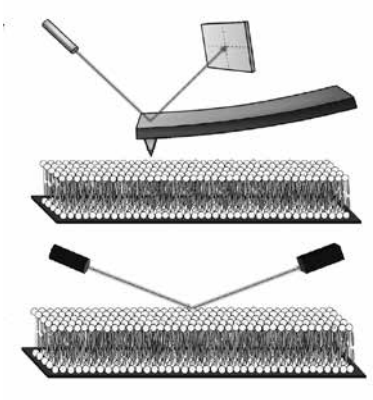
When bacteria such as *Staphylococcus Aureus* form biofilms, their resistance is enhanced, and removal of the biofilm is far from straightforward. In relation to this problem, nanomedicine is emerging as a possible approach to fight biofilm associated infections. Many strategies such as those based on liposomes, the most studied drug delivery system, still have some limitations in stability and flexibility. To overcome these limitations, Quatsomes, which are stable vesicles composed of quaternary ammonium surfactants and sterols in an equimolar ratio, have been recently developed [1]. The structural and mechanochemical characteristics of these systems are key to ensure their effectiveness as drug carriers and antibacterial agents, as in topical drug delivery the penetration enhancement capability seems to strongly depend on the nanosystem deformability. On this subject, we investigate in this work

Cholesterol/CTAB and Cholesterol/Cetrimide supported Quatsome membranes by X-Ray Reflectivity (XRR) and by Atomic Force Microscopy (AFM) and AFM Force Spectroscopy (AFM- FS) [2]. We determined, for the first time by XRR, the structural parameters of both systems and we evidence their fluid-like behavior, which is a prerequisite to obtain flexible vesicles. We report by AFM-FS that the membrane mechanical characteristics at the nanoscale (thickness, breakthrough force and dynamics) are dependent on the hydrophobic tail length of the quaternary ammonium surfactant, derived from the comparison of Chol/CTAB [3, 4] and Chol/Cetrimide membranes. Overall, these results demonstrate the tunability of Quatsome nanomechanics according to the molecular structure of its components, and place Quatsomes as promising candidates for deformable and flexible nanovesicles for drug delivery.



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Microfluidic technology for real-time imaging of drug delivery systems stability and extravasation

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Drug delivery vehicles confront multiple physiological barriers afterinjection into the human body. Their performance strictly depends on the stability in that complex environment. Effective nanocarriers should be stable to successively overcome the encountered barriers avoiding premature drug release, while being smart tofree the cargo once the target is reached. Ideally, the performance of a drug nanocarrier should be evaluated in an environment mimicking human physiology, to reduce and refine the number of preclinical and clinical trials. Commonly used 2D cell culturemodelsdo not reflect the dynamic and complex organization of a human body, meanwhile animal models are ethically arguable, expensive, time-consuming and still lack direct translation due to the differences between species. Those challenges in drug delivery screening,together with the emerging era of microfluidic technology are the driving forces for the creation of new solutions [1,2].

In our work we present a perfusable 3D cancer-on-a-chip platform, where we study time-and space-resolved stability of potential drug delivery nanocarriers. In the microfluidic chip we recreated a part of tumor microenvironment, where we focus on essential barriers challenging drug delivery systems stability. Themodel recapitulates present in vivoperfusable blood vessel lined with organized Human Umbilical Vein Endothelial Cells(HUVECs),that create the cellular wall between the vessel lumen and the extracellular matrix, in which 3D HeLa cancer cells spheroids are distributed [3].

We perfused the microfluidic platform with amphiphilic PEG-dendron hybrids (previously studied in 2D cell culture), that change their fluorescent properties upon assembly into micelles[4]. Thanks to the compatibility of the microfluidic model with confocal spectral imaging we could register real-time stability and extravasation of the introduced nanostructures interacting with the 4 defined barriers (blood vessel, endothelial wall, extracellular matrix, tumor spheroid).

We observed a difference in extravasation of nanostructures depending on theleakiness of the endothelial barrier (studying healthy blood vessel and tumor blood vessel models), what correlates to the Enhanced Permeability and Retention (EPR) effect found in vivo. Furthermore, we were able to identify most and least stable formulations by following their fluorescence emission. We registered decreased micelle internalization comparing to previously investigated 2D cell culture.Our results demonstrate the applicability of the cancer-on-a-chip platform in bridging the gap between 2D and in vivostudies. The vision of fast screening of a drug nanocarrier candidates in a 3D cell culture that could use patient derived cancer cells provides knew knowledge in the field of nanomaterials' performance and brings us one step closer to the personalized nanomedicine.

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Antimicrobial and non-adhesive electrospun wound dressings of polycaprolactone

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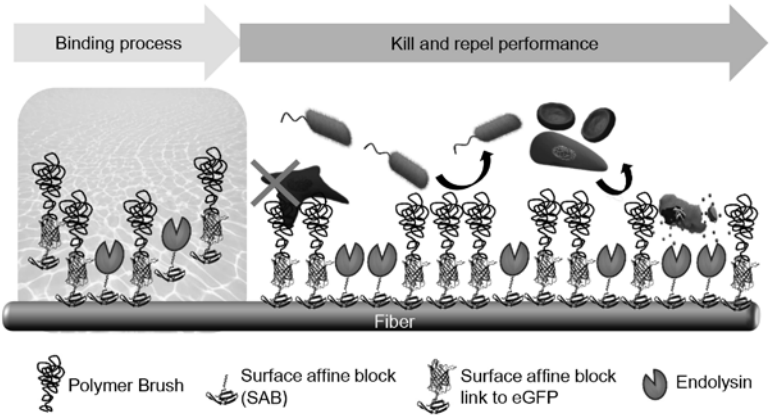
Undesired adsorption of proteins and cells on wound dressings impairs wound healing by triggering foreign body reaction and conglutination to the wound bed, causing severe damage during dressing removal. A prominent obstacle to overcome are wound infections, in particular biofilm formation. We propose a non-adhesive wound dressing with antimicrobial action not only preventing bacteria from colonization but also exhibiting bactericidal activity.

Surface activity of the antimicrobial peptide (AMP), liquid chromatography peak I (LCI), is exploited to achieve irreversible binding of protein-polymer and protein-enzyme hybrids to the surface of polycaprolactone (PCL) electrospun fibers via physical interactions. Antifouling properties are introduced by growing polymer chains directly from the AMP utilizing single electron transfer-living radical polymerization, thus developing protein-polymer hybrids. The polymerization did not affect the peptide's integrity as it was demonstrated by circular dichroism. The polymer chains consisting of carboxybetaine methacrylamide and N-hydroxypropyl methacrylamide were synthesized from aqueous solution (PBS). Bactericidal activity is achieved by fusing the AMP with the bacteriolytic enzyme endolysin which cleaves the bacteria's cell wall at specific sites but does not harm eukaryotic cells. Electrospun PCL fibers are functionalized by immersing them into a mixture of peptide-polymer and peptide-endolysin hybrids.

The assembly of hybrids on surfaces was studied by surface plasmon resonance and followed a Langmuir isotherm. X-ray reflectivity showed two distinguishable layers on the surface corresponding to the AMP and polymer chains, thus indicating surface-oriented adsorption of the LCI peptide. Remarkably, the coated PCL fibers were capable of preventing adsorption of proteins as well as adhesion of fibroblasts and the pathogen *E. coli*. The bactericidal activity of the coating systems was demonstrated by *in-vitro* experiments showing the reduction of viable cells upon contact with nanofibers. Our wound dressings are capable of preventing the colonization by pathogens and conglutination with the wound site. We propose a system with outstanding versatility, ease, and mild production. This establishes a universal method



without affecting the fiber structure.

Figure 1:Conccept image of the coating system showing the immobilization of the protein-polymer and protein-enzyme hybrids to the surface of polycaprolactone (PCL) electrospun fibers via physical interactions in aqueous solution. The coating system endows PCL fibers with antifouling and bactericidal properties.

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Novel mRNA vaccination strategies: use of poly(beta aminoesters) to achieve selective dendritic cells delivery

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Advances in immunotherapy highlight the importance of the immune system as a valuable factor in the treatment and cure of a wide range of diseases, including cancer and infectious diseases. Given the paradigm with the current crisis triggered by coronavirus disease, the development of vaccines is more than ever a crucial research goal. Vaccine design has evolved towards the use of genetic material, particularly mRNA, as the active principle to activate the patient's immune system. However, the labile nature of this molecule represents a major obstacle, as the encapsulation of RNA is required. Besides, the mRNA must target specific cell populations, usually Antigen Presenting Cells (APCs), to activate the immune response. Thus, it is necessary to design vectors for efficient delivery of the oligonucleotides. Here, we highlight the excellent results obtained in our group regarding the design of poly-beta amino ester nanoparticles. These vectors, easily tunable for the encapsulation of genetic material and adjuvants, stand out for their versatile nature in terms of application, being easily scalable under GMP conditions.

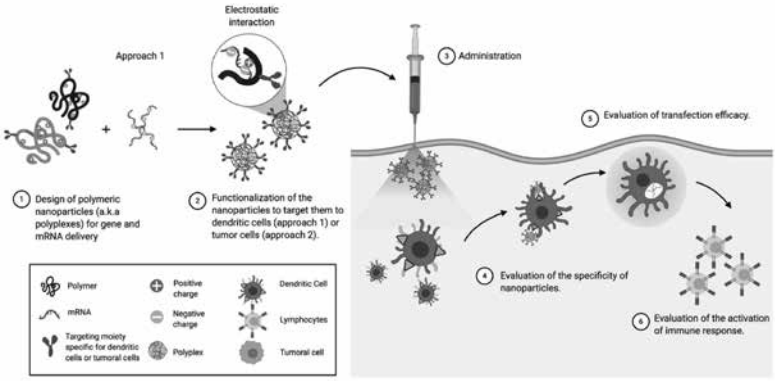

Figure 1. Graphical Abstract

A new strategy for nanoparticle brain targeting by selectively labelling the brain micro-vasculature

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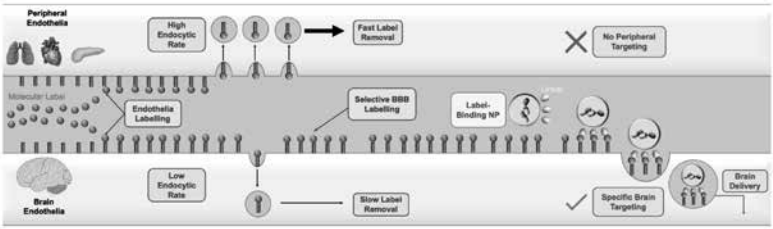
Keywords: Brain targeting, BBB, nanoparticles, endothelial labelling

Nanoparticle (NP) brain-delivery proteins strategies targeting overexpressed at the brain micro- vasculature (e.g. TfR1, Glut1) have substantial specificity limitations due to significant protein expression in peripheral organs[1,2]. We have developed[3] a new strategy to target NPs to the brain with increased specificity by instead selectively labelling the brain microvasculature. We exploit the lower constitutive endocytic rate of brain endothelial cells[4,5] to promote retention of free targeting ligands (label) selectively on the surface of thebrain microvasculature. NPs capable of recognizing the endothelial label are subsequently administered, resulting in enhanced interaction with and accumulation in the brain without increased peripheral uptake (scheme 1). We demonstrate the feasibility of this strategy by synthesizing avidin-functionalized polymeric nanomicelles (Av-NM) (fig. 1) capable of recognizing biotin- labelled endothelial cell surfaces. We show Av-NM are specifically internalized *in vitro* into brain endothelial cells following cell surface biotinylation (fig. 2a-c). Furthermore, we demonstrate generation of brain targeting specificity *in vivo* by enhanced retention of a biotin- a-PECAM1 antibody ligand on the brain vs. lung, heart and pancreas microvasculature, resulting in specific targeting of Av-NM to the brain *in vivo* (fig. 3a-f). The present work therefore provides the basis for a new targeting strategy which shifts from functionalizing NPs with ligands targeting proteins over-expressed at the brain micro-vasculature, to instead exploit the phenotype of brain endothelial cells to generate the required NP targeting specificity by selectively labelling the brain microvasculature. Hence, this work opens possibilities for new target identification based on differential endocytic rates to achieve truly specific brain NP targeting without enhanced accumulation in peripheral organs.



***Scheme 1**. Exploiting the lower endocytic rate of brain endothelium to selectively label the brain microvasculature for targeted NP delivery*

Figure 1. Avidin-functionalized polymeric nanomicelle synthesis and characterization through DLS and TEM

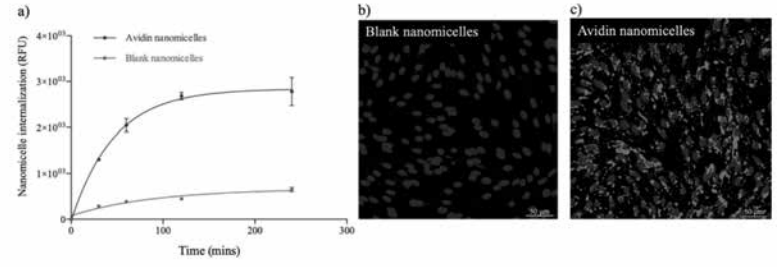
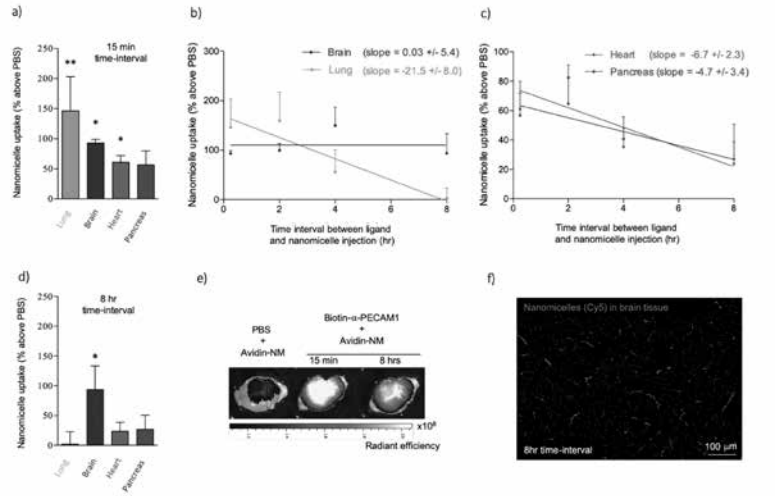


Figure 2. Specific internalization of avidin-functionalized nanomicelles into brain endothelial cells by cell surface



biotin labelling *in vivo*

Figure 3. *In vivo* brain targeting of avidin-functionalized nanomicelles (Av-NM) by selective retention of a biotinylated anti-PECAM1 antibody (biotin-a-PECAM1) ligand on the surface of brain endothelial cells. Mice were injected with free, unconjugated biotin-a-PECAM1 followed by injection of Av-NM at increasing time intervals.

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Nanobots Towards Bladder Cancer Theranostics: From *In vitro* Targeting to *In Vivo* Imaging

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Bio-catalytic nanomotors hold great promise in nanomedicine, showing improved diffusion and navigation within biological environments using endogenous fuels, [1] as well as superior drug delivery and interaction with biological structures compared to non-motile nanoparticles [2-4]. We have recently demonstrated that actively moving urease-powered nanomotors modified with antibodies against the Fibroblast Growth Receptor [3] exhibit improved targeting capabilities against 3D bladder cancer spheroids. In addition, we observed that these antibody-modified nanomotors induced higher suppression of spheroid proliferation than bare nanomotors, which could be due to the combined effect of the inhibition of the fibroblast growth factor pathway by the antibody and the local production of ammonia by the active nanomotors.[4]

However, an efficient clinical translation of nanomedicines require not only a large number of nanoparticles, but also a degree of cooperativity on their action to achieve high effectiveness.[5] This demands for a better understanding, control and visualization techniques for nanoparticle swarms in order to best evaluate motile nanomedicines and facilitate clinical translation. In this regard, we studied swarms of self-propelled enzyme-nanomotors *in vitro* and *in vivo*, as well as the effect of collective motion on the nanomotors distribution within mice's bladders. *In vitro* experiments using optical microscopy and positron emission tomography (PET) revealed enhanced fluid mixing and collective migration of nanomotor swarms. *In vivo*, intravesical administration of motile nanomotors in mice resulted a homogenous distribution of nanomotors within the bladder, while control passive particles showed sustained phase separation inside the bladder after the entrance of fresh urine.[5]

Altogether, these results demonstrate that the bio-catalytic decomposition of urea can provide urease nanomotors with active motion, enhanced targeting capabilities, as well as convection and mixing capabilities within living reservoirs, paving the way for the use of nanorobotics in theranostic applications.

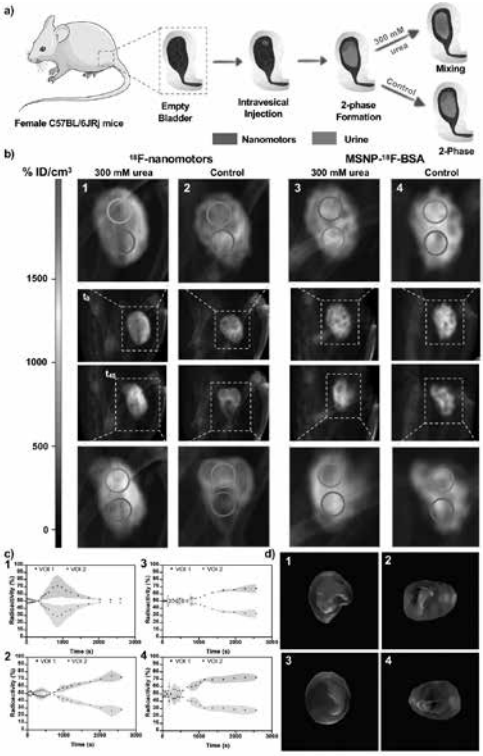

Figure 1. PET-CT analysis of the biodistribution in the bladder of radiolabeled nanomotors and radiolabeled BSA-coated nanoparticles administered via intravesical instillation. a) Scheme depicting the administration method and phenomena observed. b) PET-CT images obtained at different time points after the intravesical instillation of radiolabeled urease nanomotors in urea (1) and water (2), and control radiolabeled BSA nanoparticles in urea (3) and water (4). c) Quantification analysis of the PET-CT images obtained. d) 3D reconstructed rendering of PET images of bladders showing the contour of the bladder (as a semi- transparent layer) and the regions of high intensity in PET images.

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Multiscale Modeling of Organic Electronic Biosensor Response

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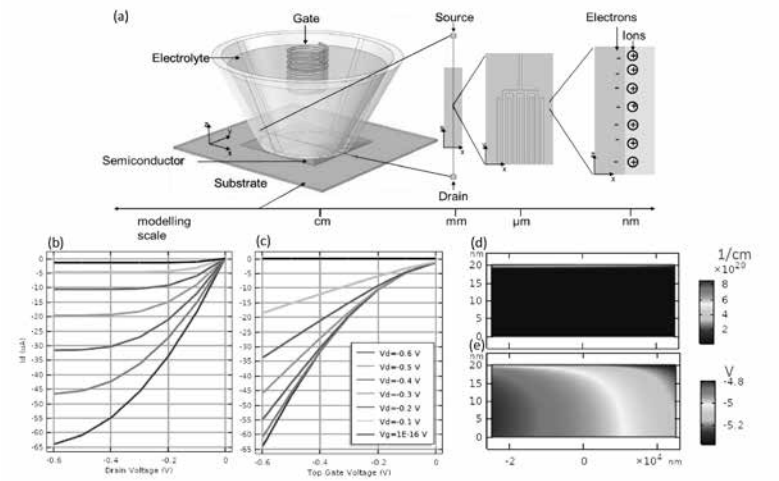
Electrolyte-gated organic field-effect transistors (EGOFETs) are being exploited as biosensors for healthcare applications due to their attractive simplicity and high sensitivity to interfacial changes, both at the gate/electrolyte and semiconductor/electrolyte interfaces [1]. In these transistors, the active channel is directly coupled to the electrolyte solution where a potential applied to the gate electrode immersed in the solution modulates the charges flowing in the transistor (Fig 1a). This configuration leads to fast, flexible and low-cost detection of drugs and diseases [2], [3]. Despite their attractive application, bringing the biosensors from research to market require further optimization (such as stability, detection limit and reproducibility), where multiscale device simulations contribute [4]. Here, we present the progress made towards the multiscale modelling of EGOFET for its use as a biosensor. We aim at coupling the drift and diffusion of ions in the electrolyte, with the drift and diffusion of holes in the semiconductor material, to investigate optimized device configurations and material parameters, and to understand the functional response of the EGOFET under different operational conditions. The results of a first model in which the electrolyte is described as a Helmholtz capacitor are presented. Current-voltage characteristics (b) and (c), and carrier and voltage distributions can be determined and show a good qualitative agreement with experimental results. The generalization of this model to include the drift and diffusion of ions in the electrolyte are discussed.



(a) Schematic setup of an electrolyte-gated transistor. (b) Output curve of thin film transistor. (c) Transfer curve of thin-film transistor. (d) Hole concentration and (e) electrical potential distribution of an electrolyte-gated OFET at VD= -0.4 V (drain voltage) and VG = -0.6 V (gate voltage).

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First steps towards mimicking cell division in fully artificial systems

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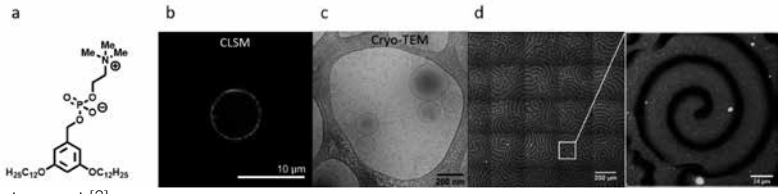
Molecules that self-assemble into cell membrane mimicking vesicles are important systems for studying natural cell processes, developing interactive systems and drug delivery carriers. Commonly employed molecules are based on natural (phospho) lipids or amphiphilic block copolymers which assemble into liposomes and polymersomes, respectively. Liposomes suffer from low mechanical and chemical stability. Polymersomes can be designed to be stable but this stability comes at the expense of much thicker and rigid bilayers compared to natural cell membranes, which are usually 4-5 nm thick. Amphiphilic Janus dendrimers (JDs) are another class of membrane-forming molecules that self-assemble into dendrimersomes (DSs) in water. Due to their modular synthesis, JDs have structural and functional variability. As a result, DSs can be designed to mimic the fundamental properties and functions of cell membranes. These vesicles display superior mechanical stability compared to liposomes while maintaining the flexibility and permeability close to the one observed in cell membranes.[1]

In this poster, we present a new type of JD which, on one hand closely resembles the structure of natural phospholipids where the hydrophilic part consists of a phosphorylcholine group, but on the other hand contains an aromatic ring in the hydrophobic part that provides additional stability to the membrane. The synthesized JD self-assembled into DSs in water, which mimicked natural cells in their bilayer thickness, their bending rigidity and their lateral mobility. We are particularly interested in programming basic activities in artificial membranes. An example of such activity is the introduction of cell divisome to control synthetic cell division. The first steps towards this goal was to form planar surface waves on a flat membrane by association of Min proteins. These proteins are crucial components of the *E. coli* divisome, as they form similar-patterns on its cell-membrane and thereby ensure that cell-division occurs along a linear plane in the middle of the cell. For this, we formed supported bilayers of these JDs to mimic the cell membrane and tested the generation of self- organized dynamic patterns after addition of MinD and MinE proteins along with ATP. When MinD was associated to the membrane it formed a homogeneous protein layer. However, when MinE and ATP were added planar surface waves formed on the membrane. This dynamic patterning was previously reconstituted on supported bilayers of natural lipids. The herein reported JD marks the first synthetic cell membrane mimic on which this dynamic pattern formation was



observed.[2]

Figure 1: The synthesized JD (a) self-assembled in water into unilamellar vesicles as observed by CLSM (b) and cryo-TEM (c). MinD, MinE and ATP self-organized into dynamic patterns on supported bilayers of the synthesized JD (d).

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Building membrane machines to endocytize living bacteria: the battle between adhesion and flexibility

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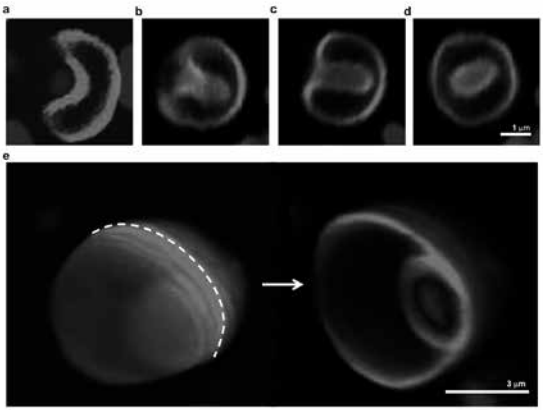
Bacterial and viral invasion into host cells depends critically on their engulfment by the plasma membrane. In cells this process, termed endocytosis, is usually active, i.e., involving components of the cell machinery, starting by the receptor–ligand engagement that triggers a series of energy- driven processes that curve cell membrane culminating in the engulfment into an endosome that is released to the cytoplasm. Since such evolved complex mechanisms yet cannot be replayed by synthetic systems, in this work we undertook the challenge of building a synthetic cell membrane-model capable of a rudimentary form of endocytosis of bacteria to address the question: What are the minimal requirements necessary for endocytosis of a bacteria?

Theoretical studies predict that the endocytosis of prolate micro-objects is possible without the need of active cell machinery if the energy released upon bacterial adhesion to the membrane surpasses the energy required to bend the membrane.[1, 2] However, current widely accepted cell membrane mimics such as liposomes and polymersomes fail to sufficiently recapitulate membrane properties to perform such complex functions. Here we report the engulfment of living bacteria into endosomes by cell-like dendrimersomes (DSs) assembled from Janus dendrimers (JDs).[3] The biggest advantage of this synthetic system is that the key properties of the DSs can be programmed into the molecular structures of their building blocks. The mechanism of engulfment included the following steps: adhesion, invagination, formation of constriction, and release of an endosome. Full engulfment occurred in less than a minute after contact with *E. coli*. The process was driven by the adhesion of the bacterium to the DSs' membrane by ultraweak interactions, comparable to those utilized by nature. The key to success relies on the combination of high flexibility and stability of the DSs. The ability to support endocytosis highlights opportunities for the



design and programming of DSs in biomedical research.

Figure 1. CLSM images show the process of engulfment of bacteria (blue) by DSs (red). (a) Adhesion of E. coli to the DS membrane. (b, c) Invagination of E. coli into the interior of the DS. (d) Formed endosome with living bacteria inside. (e) 3D reconstruction of 150 confocal scans for whole (left) and 80 confocal scans for half (right) of the DS with engulfed E. coli. The white dashed line on a whole DS indicates the place ofintersection for the presentation of half of the DS to show the interior of an endosome with engulfed bacteria.

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Electron Transport Study in Olfactory Receptor 1A1

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Olfactory receptors (ORs) comprise the largest multigene family in the mammalian genome. They belong to class A rhodopsin-like family GPCRs (G-protein coupled receptors)[1], and are expressed in the cell membrane of olfactory neurons. Although there are no resolved structures for any ORs, sequence homologies have been found with opsin[2].

A characteristic of ORs is the promiscuity they show in ligand binding by displaying affinity for a range of odorant molecules, and a single odorant molecule able to bind several olfactory receptors with varying affinities. Therefore, the induced fit model generally accepted for ligand binding in GPCRs, seems inaccurate to describe odorant recognition by ORs. Other models have been proposed. The vibrational theory of olfaction (VTO)[3,4] assumes there is a correlation of the vibrational modes of the ligand in the Raman spectra with odor (odor given by chemical bonds)[5]. Nevertheless, VTO found exceptions and generated a lot of controversy[6,7].

The VTO model has been further elaborated and a mechanism has been proposed in which the correlations found between Raman spectra of the ligands and odor can be attributed to inelastic electron tunneling (IET) occurring at the binding site[8,9]. Proteins are quite efficient electronic conductors and their electron transport (ETp) characteristics have been addressed in many studies[10]. The process of ETp assisted by collective vibrations in a protein has been documented, as well as the activation of the vibronic properties in the active site of a protein caused by inelastic electron transfer[11]. Here we studied ETp in human olfactory receptor 1A1 (hOR1A1) by electrochemical scanning tunneling spectroscopy (EC STS) in the presence and absence of the ligand dihydrojasmone. We observed that, although no significant changes were found in the conductance of hOR1A1 obtained from current-voltage (IV) and break-junction experiments, the presence of dihydrojasmone induced a shift in the voltage of open circuit (VOC) measured in IV characteristics.

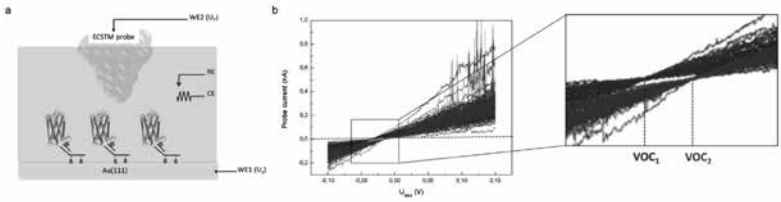

Figure. Electron transport (ETp) in human olfactory receptor 1A1 (hOR1A1) studied with electrochemical scanning tunneling spectroscopy (EC STS). a) Schematics of the electrochemical scanning tunneling microscope (EC STM) set-up. hOR1A1 was overexpressed in a mammalian unducible cell line[12]. The purified receptor was immobilized on Au(111) substrates through half anti- Rhodopsin against the C-terminal tag Rho1D4 (TETSQVAPA). b) Current-voltage (IV) curves recorded for h1OR1A1 (blue traces) and h1OR1A1 with dihydrojasmone ligand (purple traces). Red dashed line indicated zero current and, in the zoomed region, the voltage of open circuit (VOC) was indicated showing a shift to lower potentials (absolute value) induced by the ligand.

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Study of a New Generation of Surgical Mesh for hernia repair: a flexible, smart and self-evolving sensor/actuator with 4D Response

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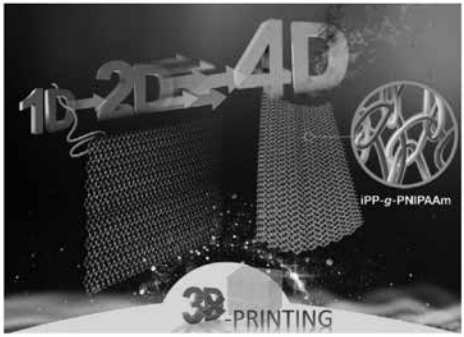
The fourth dimension (4D) concept for creating dynamic devices, that can change their shape and/or function under controlled stimulus, is an emerging technology [1]. Here, a smart material, able to self- unfold under temperature and humidity stimula, has been developed. For the first time, this study reports the 4D movement of a flat surgical mesh, employed for hernia repair (Figure 1) [2]. By means of a facile approach, a smart actuator composed by a substrate of fibers of isotactic polypropylene (iPP) mesh and a coating of thermosensitive poly(N-isopropylacrylamide) (PNIPAAm) hydrogel, is obtained [3,4]. Thermo- responsive poly(N-isopropylacrylamide) (PNIPAAm)-based materials are widely applied in biomedical field owing to their excellent biocompatibility and abrupt conformational change at a critical temperature very close to that of human body (~32 °C)[5].

The thermo dependent motion of the hydrogels chains is responsible for the smart behaviour of the mesh: i) it simultaneously works as an actuator, able to self-unfold after implantation in human body, improving the manage from the surgeons, and ii) as a sensor that can detect local temperature increase due to inflammatory processes. The macroscopic and microscopic stimulus response was evaluated through an infrared thermographic camera and by optical microscopy. The PNIPAAM gel expansion/contraction as well as the time of folding/unfolding response were approached. The dimensional stability and the mechanical integrity of the coated mesh were also investigated. It must fulfil with the mechanical properties of abdominal wall requirements need for hernia repairs.. To this purpose, the bursting strength of the iPP-g-PNIPAAm meshes was evaluated, showing an increase of ≈16% with respect to the uncoated mesh, offering a strongest and adaptable system for its



future implantation in human body.

Figure 1. Illustration of the fourth dimension (4D) concept for the iPP-g-PNIPAAm mesh.

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Unidirectional Photosynthetic Complex functionalization for tunnel current distance decay spectroscopy

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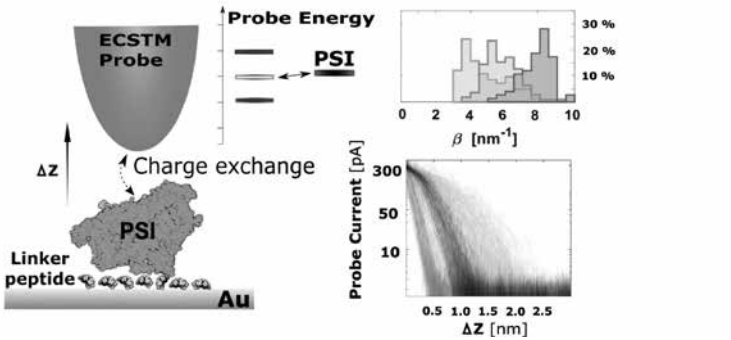
Interprotein electron transport (ET) is a key process for living organisms, playing a fundamental role in respiration and photosynthesis. ET processes between photosynthetic complexes and electron carrier partner proteins have been widely studied with bulk spectroscopic techniques[1], however the characterization of ET at the level of single molecules is limited. This is due in part to the lack of a well- defined experimental setup for protein orientation and current measurement. In this work, a peptide[2] that binds selectively to plant Photosynthetic Complex I (PSI) in is used to functionalize atomically flat gold monocrystal electrodes and to evaluate photocurrents by bulk photo-electrochemical experiments and ET by scanning probe techniques, atomic force microscopy (AFM) and electrochemical scanning tunneling microscopy (ECSTM). ECSTM-based spectroscopic measurements allow investigating the current decay distance[3] (β [nm^{-1}]) of PSI functionalized electrodes under electrochemical control. Mapping β over sample and probe potentials reveals enhanced charge exchange distance as probe potential is aligned with PSI's electron acceptor cofactor redox potential.



Left: Cartoon representation of peptide-PSI molecules absorbed on Au substrates in ECSTM experiment. Energy diagram displaying probe fermi level (green bar aligned, blue bars misaligned) and PSI ET cofactor redox potential (black bar). Right bottom: Semilogarithmic plot of tunnel current vs probe distance for aligned (green), misaligned (blue) probe potentials in peptide-PSI functionalized electrodes and bare Au control (yellow). Right Top: Histogram displaying current decay distance (β [nm-1]) distribution for curves shown bellow.

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Azobenzen/nitrazepam-based light-controllable modulators of inhibitory brain receptors

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Inhibitory neurotransmission in the brain is mediated mainly by two ligand gated receptors – GABA and GlyRs. Disequilibrium in their function leads to many severe neurological disorders, such as epilepsy, hyperekplexia, anxiety, depression, schizophrenia and sleep disorders. Thus, development of allosteric modulators that would regulate the activity of these receptors with minimized side effects is of great importance. Photopharmacology is a unique tool for these purposes, allowing precise spatial and temporal light-driven control of pharmacophores' activity, and consequently of their target proteins. Here we present a series of azobenzene-based derivatives of benzodiazepine that successfully photocontrol activity of GABA and GlyRs. Compounds obtained by azologization of 7-aminonitrazepam with nitrosobenzenesulfonamide and nitrosopyridine (UR-DW285 and UR- DW290 respectively) demonstrated reversible photochromism and were selected for *in vitro* testing at inhibitory receptors. GABA and GlyRs were heterologously expressed in cultured CHO cells, their activity was monitored using whole-cell configuration of patch-clamp technique. At visible light UR- DW285 inhibited activity of GABAA,C and alpha2 GlyRs, while upon UV (365nm) irradiation their activity was restored. Using series of point mutations and molecular modelling approach we were able to demonstrate that UR-DW285 is a photo-switchable ion channel blocker of $GABA_{A,C}$ and GlyRs. UR-DW290 displayed minor activity at GABARs, but it allowed photosensitive modulation of GlyRs of various subunit compositions. In *trans* configuration UR-DW290 slightly inhibited glycine-induced currents, while in *cis*, generated by UV illumination, inhibitory effect was significantly strengthened. Zebrafish testing demonstrated high efficiency of UR-DW290 for the photo-control

of their inhibitory system. In summary, we have developed a group of azobenzene/nitrazepam based compounds that modulate activity of two main inhibitory brain receptors in light-dependent manner. Contrary to our expectation they did not potentiate GABAR currents but induced subunit-specific inhibition of both GABA and GlyRs. UR-DW290 that specifically interacts with GlyRs was shown to be efficient for light-dependent modulation of zebrafish behavior *in vivo*.

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Anti-fouling hydrogel coatings for medical devices

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The quality of life for many people suffering from dysfunctions involving the damage of tissue or organs is dramatically improved by the use of indwelling medical devices, such as various implants or catheters. However, the major drawback of these devices is the adhesion of proteins and bacteria to their surface that leads to the formation of biofilm affecting the lifetime of the device and ultimately increasing the risk of biomaterial-centered infections. So far, the most effective system that can repel proteins and bacteria are polymer brushes. However, the synthesis of anti-fouling polymer brushes requires complex surface-initiated polymerization techniques that are difficult to translate to industrial production. It remains a great challenge to design a versatile and efficient coating technique that exhibits anti-fouling properties similar to polymer brushes while still being easy to handle in terms of synthesis and application.

Here, we report anti-fouling hydrogel coatings from hydrophilic poly(oligoethyleneglycol methacrylate) (poly(MeOEGMA)) and zwitterionic poly(carboxybetaine methacrylamide) (poly(CBMA)) functionalized with photo-active benzophenone moieties that can be applied to a wide range of polymeric biomaterials and prevent the undesired adsorption of proteins or bacterial adhesion. The incorporation of benzophenone by statistical copolymerization with benzophenone methacrylate or methacrylamide enables these polymers to be linked covalently to any surface containing carbon-hydrogen bonds within a few minutes via UV-induced C, H- insertion resulting in the formation of a highly hydratable surface-attached polymer network. The coatings were applied to common polymeric materials that are widely used for the fabrication of biomedical devices, like polymethylpentene (PMP) and polyethylene (PE). All coatings were able to reduce the adsorption of proteins from blood as well as the adhesion of *E. coli* compared to unmodified substrates.

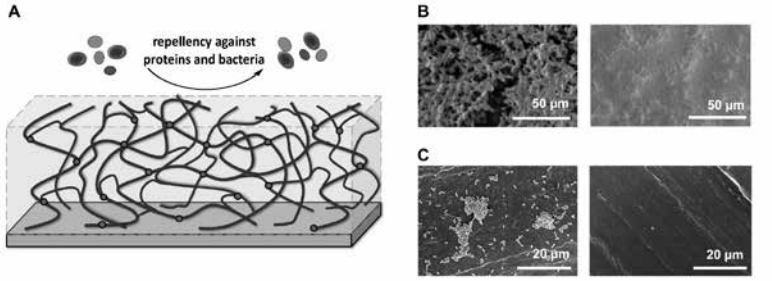

Figure 1: A) Schematic illustration of a surface-attached hydrogel coating. B) Scanning-electron microscopy (SEM) images of unmodified PMP and PMP coated with MeOEGMA-based hydrogel after incubation with heparinized full blood for 2 h. The bare PMP surface rapidly induces coagulation after contact with blood and is covered with a fibrin network (left), while the PMP substrate modified with the hyrogel coating exhibits a clean surface (right). C) SEM images of unmodified PE substrate and PE coated with CBMA-based hydrogel after incubation in e. coli bacterial suspension (CFU = 107) for 24 h. While the unmodified PE substrate is covered with bacterial cells (left), the PE substrate modified with the hydrogel coating remains clean (right).*(2015-FI-B-00817).*

Mapping the Capacitance of Self-Assembled Monolayers at Metal/Electrolyte Interfaces at the Nanoscale by In-liquid Scanning Dielectric Microscopy

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The electric polarization properties of ultrathin layers at the metal/electrolyte interface can play a fundamental role in many interfacial electrochemical processes. Currently, there is a lack of techniques able to address these properties with nanoscale spatial resolution. Here, we show theoretically, in the framework of the Nerst-Planck-Poisson theory, and experimentally, on micropatterned self-assembled monolayers, that the dielectric properties of ultrathin layers with thicknesses below 1 nm can be imaged and quantified at the nanoscale by means of in-liquid Scanning Dielectric Microscopy (1), (2) combined with finite element numerical calculations. We demonstrate that a spatial resolution down to ~100 nm can be achieved. The extreme sensitivity of in-liquid Scanning Dielectric Microscopy to the dielectric properties of ultrathin films could offer novel insights on the nanoscale electrochemical properties in fields as diverse as biosensing, organic bioelectronics, bioelectricity, biochemistry or energy storage. Present results open the access to study the dielectric properties of metal/thin film/electrolyte interfaces at scales that have remained unexplored until now (3).

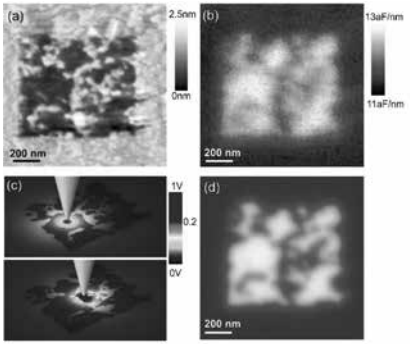

Figure 1: (a) AFM topographic and (b) constant height (z=12 nm) SDM capacitance gradient images of an imperfectly scratched ~1 nm thin dodecanethiol SAM on a gold substrate in a low conductivity aqueous solution. (c) Electric potential distribution on the surface of the SAM layer numerically calculated for a tip of small radius R=25 nm and angle θ=18o at two different positions of the tip. (d) Simulated capacitance image.

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Multi-ligand targeting nanoparticles for personalized cancer therapy

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Personalized medicine is an emerging practice of medicine that aims to tailor treatments to fit each patient's unique disease molecular features. Recent advances in the medical applications of nanotechnology have allowed for the incorporation of multiple therapeutic, diagnostic and targeting agents into nanoparticles (NPs), offering a new ray of hope for detection, prevention, and treatment in oncology. The application of nanomedicine for development of personalized treatment approaches holds great potential to revolutionise the field of cancer therapy. Presently, patients treated with conventional chemotherapy suffer from side effects of the drugs due to their non-selective action on healthy cells.

Active targeting using NPs conjugated with targeting ligands on the surface play an important role in improving drug selectivity to cancerous cell. Cancer cell targeting peptides (CTPs) are known to be specific for cancer-related surface markers, such as membrane receptors, and can be used to deliver cytotoxic cargo specifically to cancer tissue or vasculature. However, several gaps still exist in our understanding of this nano-bio interaction, and also the extent of specificity of CTPs to be able to exploit their potential for designing more efficient and targeted nanocarriers, thereby calling for the use of powerful imaging techniques to better characterize them.

Within this framework, the present study is designed to characterize the specificity of CTPs known to bind to certain prostate cancer cell receptors: Prostate specific membrane antigen (PSMA) and epithelial growth factor receptor (EGFR). The CTPs chosen are synthesized manually and conjugated to polymeric PLGA-PEG nanoparticles. The active targeting potential of these NPs is explored using confocal laser scanning microscopy and flow cytometry. Further, we intend to explore the effect of using multiple CTPs on selective targeting of prostate cancer cells. The knowledge obtained from this kind of study intends to help generate a library of nanoparticles with multiple targeting peptides used in different ratios to develop effective nanocarriers for individual cases, serving as a mean for personalized nanomedicine.

Judging micelles by their covers: combining molecular precision and self- reporting mechanism for a direct comparison of dendritic amphiphiles with different hydrophilic blocks

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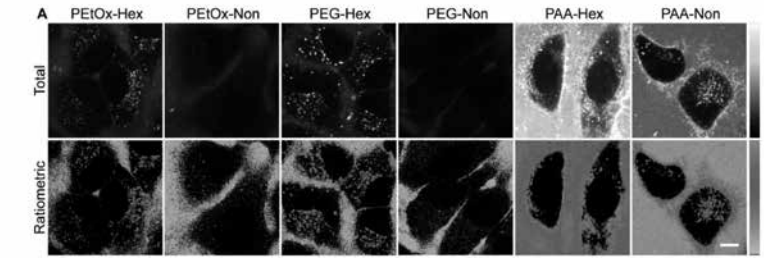
Enzymatically cleavable polymeric micelles are self-assembled nanostructures made of polymeric amphiphiles that show great potential as drug delivery systems due to their hydrophobic core that can encapsulate lipophilic drugs. Creating an enzymatically cleavable design of the hydrophobic core has many advantages, such as better control of drug release and faster clearance of the remaining amphiphiles. While extensive research focuses on the design and comparison of hydrophobic blocks, the effect of the hydrophilic shell on micelle structure is not well understood. In this study, we compared three widely used hydrophilic polymers, namely polyethylene glycol (PEG), poly (2-ethyl oxazoline) (PEtOx) and polyacrylic acid (PAA) in order to determine their effect on micelle properties, including drug loading capacity and stability in biological media. As hydrophobic cores we used dendrons, for having well-defined structures with adjustable degree of hydrophobicity, containing either 6 or 9-carbon chains respectively. As means of reporting, a coumarin fluorescent dye was inserted for producing a spectral shift between the assembled and disassembled state. Micelles were thoroughly characterized in terms of size, CMCs, enzyme response, drug loading, stability in biological media and interaction with cells. Having well-defined hydrophilic and hydrophobic components, we were able to distinguish the effects that each of them play on different micelle characteristics. Thus, we found that micelle size depended mostly on the hydrophobic block, while enzymatic degradation was strongly influenced by both components. Drug encapsulation capacity, as well as internalization in HeLa cells and drug release, were highly dependent on the type of hydrophilic block. Thus, we concluded that

the choice of hydrophilic shell is very important for the efficacy of micelles as drug carriers. Overall, the high molecular precision of the studied amphiphiles and the ability to report their disassembly even in complex biological media, allowed us to directly compare different types of micelles, providing striking insights into how the composition of the hydrophilic shell and the hydrophobic core of polymeric micelles can affect their properties and potential to serve as nanocarriers.

**These authors contributed equally*



Development and permeability evaluation through BBB-on-a-chip model of Gold nanorods with therapeutic potential for Alzheimer's disease

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Alzheimer's disease (AD) is a chronic neurodegenerative disorder characterized by a progressive loss of cognitive capacity and memory. AD is mainly associated to the accumulation of toxic aggregates of amyloid β peptide (Aβ) in the brain that produce oxidative stress and neurotoxicity [1]. In the last years, multiple efforts have been performed in order to develop new molecules for AD's treatment based on the disaggregation of Aβ cumulates [2]. However, most of them do not reach the action site due the strict permeability in the brain by the blood brain barrier (BBB).

Nanotechnology is a cutting-edge field that extends different possibilities for the diagnosis and treatment of AD. In this direction, a nanosystem for AD treatment was reported that consists in gold nanorods (GNRs) functionalized with polyethylene glycol (PEG), a β sheet breaker peptide (D1) and a peptide to shuttling through the BBB (Angiopep-2). The results revealed that the GNRs-PEG-Ang2/D1 nanosystem inhibited Aβ growth *in vitro* and decreased the toxicity of Aβ aggregates in an *in vivo* model [2].Therefore, promising therapy agents are being developed and it is required to be evaluated quickly and easily for the early provision of new alternatives for AD. BBB-on-a-chip is an interesting platform due their versatile, controlled and lower cost design to mimic both *in vivo* physiological and pathological conditions for the study of drug permeability, disease progression and treatment efficacy [3].

In this work, we synthetized and characterized GNR-PEG-Ang2/D1 by absorption spectrophotometry, dynamic light scattering, laser Doppler micro-electrophoresis and transmission electron microscopy. Then, BBB-on-a-chip device was fabricated consisting in a neural chamber with human astrocytes and pericytes and a lateral channel with human brain endothelial cells in order to mimic the BBB. We determined the cytotoxic effect of GNR-PEG- Ang2/D1 over the above mentioned cells. Finally, the permeability of the nanosystems was evaluated through the BBB-on-

a-chip device by confocal microscopy. The results confirm that GNR-PEG-Ang2/D1 was successfully synthetized and functionalized with the peptide Angiopep- 2 and D1. In addition, GNR-PEG-Ang2/D1 showed non-toxic effect for the tri-culture at the given range of concentration for 24 hours. BBB-in-a-chip results showed the development of tight junctions between the adjacent endothelial cells in the chip, which are crucial for permeability assays. Lastly, GNRs permeability assay revealed differences between the chip control and the chip exposure to GNRs by confocal microscopy. Consequently, BBB-on-a-chip could be a functional and throughput platform to evaluate the nanoparticles permeability.

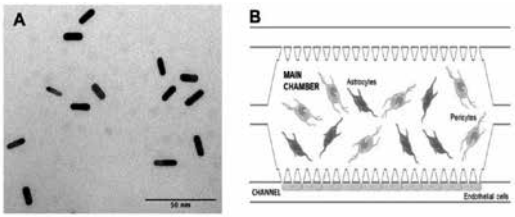

Figure 1. *(A) Electron micrograph of GNRs-PEG-Ang2/D1 (B) Cell arrangement of tri-culture into BBB-on-a-chip.*

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Controlling the Activation of Coagulation at Interfaces

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Is it possible to control coagulation at interfaces by creating a surface which locally inhibits the contact activation system and prevents clotting without compromising hemostasis?

Contact between any surface of medical devices and blood inevitably induces coagulation by adsorption and surface-activation of factor XII (FXII) (contact activation), followed by the activation of platelets and leukocytes on the surface. This event triggers thrombotic complications in patients and leads to failure of the device. The current strategy to overcome this problem is to systemically administer anticoagulants which target and inactivate key enzymes in the later stage of coagulation. However, this treatment not solely impedes clot formation but also disrupts hemostasis, resulting in a higher risk of hemorrhage accompanied by increased patient mortality. To overcome the obstacle of insufficient hemocompatibility we developed an ultra-thin coating, which controls coagulation at different levels. The first level (passive level) was to prevent unspecific protein adsorption from blood and activation of coagulation. For this, a layer of hydrophilic copolymer brushes was grafted from a model surface via SET-LRP. In the second step, we implemented an additional mode of action by covalently immobilizing different biomolecules to the brushes. Thus in case some activation occurs the coating autonomously reacts and inactivates key enzymes (FXIIa, FXIa, kallikrein) of the intrinsic coagulation pathway (interactive level) while not hampering the overall hemostasis.

Antifouling studies by surface plasmon resonance showed that the polymer brushes were repellent on a molecular scale and prevent the unspecific adsorption of FXII and blood plasma proteins. We further challenged substrates coated with polymer brushes with human whole blood and analyzed the samples by scanning electron microscopy (SEM). No platelets and cells adhered to the surface prooving the additional long-range repellency of the brushes on a mesoscale. The immobilization of human antibody (anti-FXIIa), corn trypsin inhibitor (CTI), soy bean trypsin inhibitor (STI), and C1-esterase-inhibitor (C1- INH) on the brushes and their ability to bind the different coagulation enzymes to the surface was assessed by SPR spectroscopy. In addition, a chromogenic activity assay was used to prove the binding capacity of anti-FXIIa immobilized on polymer brushes grafted from polycaprolactone fiber mesh. FXIIa specifically cleaves the chromogenic peptide and releases an UV-detectable dye. When the anti-FXIIa coated fibers were present in solution the cleavage of the chromogenic substrate was completely prevented up to concentrations of 30 nM FXIIa in solution.

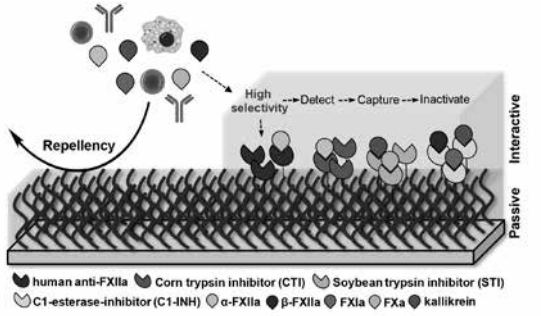

Figure 1: Concept of the bioactive antifouling coating. The bottom polymer brush layer (passive) is stealth to blood and prevents adsorption and unfolding of proteins, as well as the adhesion and activation of platelets and factors. The top level is the interactive level showing the different immobilized inhibitors. Key enzymes of coagulation are specifically captured and inactivated thereby modulating coagulation at the interface.

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Development of DNA aptamers against enzymes of the methylerythritol phosphate pathway

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Malaria is a disease caused by parasites of the genus *Plasmodium* and transmitted by female *Anopheles* mosquitoes. According to the most recent data, it is estimated that 228 million cases of malaria occurred worldwide in 2018, which led to 405,000 deaths[1].Due to the resistance acquired by *Plasmodium* to antimalarial drugs, the global technical strategy against malaria (World Health Organization, Strategic Plan 2016-2030)contemplates the search for solutions to the threat of the emerging resistance to antimalarials[2].

Plasmodium parasites have an organelle called apicoplast, which offers numerous new targets for drug therapy because it contains a range of metabolic pathways and enzymes not found in human cells[3]. Of these pathways, the enzymes required for biosynthesis of isoprenoid precursors, isopentenyl diphosphate and its isomer dimethylallyl diphosphate are particularly attractive because they are essential for the survival of the parasite. These precursors are produced in *Plasmodium* from pyruvate and D-glyceraldehyde 3-phosphate via the methylerythritol phosphate (MEP) pathway[4]. Thus, as an antimalarial therapeutic alternative, we propose the development of aptamers inhibiting key enzymes of the MEP pathway.

Several methods have been optimized, such as: (i) the production of MEP pathway enzymes, (ii) the development of aptamers by Systematic Evolution of Ligands by Exponential Enrichment, (iii) the cloning of aptamers, (iv) the establishment of an Electrophoretic Motility Shift Assay for the identification of interactions between selected aptamers and their target enzymes, and (v) methods for the *in vitro* evaluation of enzymatic activity. Among the most outstanding results, we have identified an aptamer that in preliminary analyses seems to interact with the enzyme 1-deoxy-D-xylulose-5-phosphate reductoisomerase, a key enzyme of the MEP pathway. Currently, we are improving the experimental design to confirm the interactions between selected aptamers and target proteins. Furthermore, working on novel selection strategies and the isolation of new aptamers against key enzymes of MEP pathway will continue.

Keywords: Malaria, isoprenoids, aptamers

ACKNOWLEDGEMENTS

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Modified phospholipid vesicles as drug delivery systems for the treatment of leishmaniasis

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The leishmaniases are a group of poverty-related parasitic diseases transmitted by sandflies and caused by protist parasites from more than 20 *Leishmania* species (WHO, 2020). Current medicines for the treatment of human leishmaniases are expensive, they have high systemic toxicity and limited efficacy (Ghorbani & Farhoudi, 2018). Our objective is to develop innovative ad hoc formulated, biocompatible phospholipid vesicles able to stably load high amounts of antileishmanial drugs (pentamidine isethionate, amphotericin B, miltefosine, paromomycin and alternative drugs such as rifampicin, curcumin or quercetin) and target them to *Leishmania* parasites. Furthermore some molecules, such as antimicrobial ribonucleases (RNases) from the RNase A superfamily (Lu et al., 2018), are currently being studied to establish if they have anti- leishmanial activity, in which case they would be loaded into vesicles to improve their efficacy.

In order to increase cell targeting, nanovesicles may be functionalized with specific ligands for macrophages, such as aptamers being developed in our laboratory able to bind infected macrophages with high affinity, or heparin (Martins et al., 2018). All the formulations are prepared by using a smart, green, and scalable method, avoiding the use of organic solvents and energy dissipative steps.

Amphotericin B (AmB), pentamidine isethionate and paromomycin-loaded vesicles have been tested against one of the Leishmania infantum developmental stages: promastigotes. Some of these formulations showed IC50 values lower than those of non-encapsulated drugs. Nanovesicles containing AmB were also tested against amastigotes, the *L. infantum* life cycle stage parasitizing macrophages. The resulting formulations are expected to be inhaled, topically applied or intravenously delivered in order to treat cutaneous, visceral, or mucocutaneous leishmaniasis. Positive *in vitro* results of the novel formulations will be tested *in vivo* and in the case of topically applied nanoformulations, also studies of skin penetration and permeation experiments will be performed by means of Franz diffusion cells.

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Study of the relative expression of ICAM-1 Ig-like domains in different cell types and disease conditions

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Targeting cell-surface receptors is critical for efficient drug delivery to body sites of interest. Previous studies have shown that intercellular adhesion molecule 1 (ICAM-1), a protein expressed by most cells under inflammation, provides good targeting for drug delivery interventions. ICAM-1 gene codes for five immunoglobulin (Ig)-like extracellular domains (D1 to D5), believed to be expressed as a variety of isoforms but never verified in human cells. Since unique isoforms could increase ICAM-1-targeting specificity tremendously, this project aimed to investigate the existence of said ICAM-1 isoforms, assessing their expression in human endothelial (brain microvascular and umbilical vein macrovascular) and neural-like cell types, using pharmacological models of inflammation and lysosomal disorders.

Quantitative PCR was used to determine the mRNA level of each ICAM-1 Ig-like ectodomain. For the first time, we describe the possible presence of ICAM-1 isoforms in human cells and their different expression depending on cell type and pathology. Unstimulated (control) endothelial and neural-like cells expressed different ICAM-1 isoforms, although this was not statistically significant for microvascular endothelial cells. These isoforms displayed domains D1 and D3, the ligand-binding regions for integrins LFA-1 (leukocyte) and Mac-1 (macrophage), respectively, with D1 being predominant for all cell types and D5 being lower for endothelial cells. TNFα-mimicked inflammation increased ICAM-1 expression in all cell types, but much less in neural-like cells and faster for microvascular endothelial cells. We also detected a decay in domain D2 expression in an acute inflammation setting for microvascular endothelial cells vs. other cell types, whereas ligand-binding domains remained highly expressed. Since D2 is required for LFA-1 binding to D1, this may facilitate Mac-1 interaction with D3, which can be reinforced by macrophage binding to fibrinogen, which in turn is known to bind to ICAM-1 D1. Expression of D4, responsible for ICAM-1 oligomerization capacity, fluctuated for both endothelial cell types along the time of simulated inflammation, whereas neural-like cells seemed restricted to isoforms without D4, for which these cells may not express ICAM-1 oligomers. While neural-like

cells overexpressed ICAM-1 under lysosomal alteration simulation with imipramine, endothelial cells were not affected by this, yet imipramine has an effect under inflammation, indicating that various pathological stimulations may additionally vary ICAM-1 isoformic expression.

In conclusion, ICAM-1 protein isoforms seem to exist in human cells, which may control precisely ICAM-1 display and function. Specific ICAM-1 isoforms could increase the targeting specificity of future translational drug delivery applications.

Enhanced binding by hierarchical self-assembly of sugars in glycodendrimersomes

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Natural cell membranes contain nano- and micro-organized domains formed by non-random association of lipids. These domains facilitate multivalent and cooperative interactions that ensure specific recognition of ligands to control functions such as communication, division, cargo trafficking, and signal transduction. Located at the cellular interface the functional domains contain glycoproteins and glycolipids. The sugar moieties provide the structural basis for affinity in protein recognition and the formation of molecular patterns on the surface. But can these patterns be controlled by the sugar type? And how do these nanoarrays affect the binding of lectins, the sugar-binding proteins? To tackle these questions we utilized novel synthetic cell membrane mimics based on self-assembly of Janus glycodendrimers (JGDs) into glycodendrimersomes (GDSs).[1] The resulting vesicles closely resemble the thickness, flexibility and lateral 2D organization of natural cell membranes.[2] These properties stem from the chemical structure, architecture, and topology of the JGDs. For this study, the JGDs are decorated with the monosaccharide mannose (Man), due to its high biological relevance. The concentration of Man was systematically reduced by oligo(ethylene oxide) within one molecule.

Using atomic force microscopy, we demonstrated that a high concentration of Man on the GDSs surface leads to a homogeneous membrane surface, while diluted Man groups can organize the sugar moieties into periodic lamellar and hexagonal nanoarrays. The nanoarray formation is driven by weak hydrophilic forces between glycans to maximize interactions. We demonstrated an increase in the biological activity of sugars upon formation of nanoarrays. Kinetic studies by surface plasmon resonance showed a 10-fold increase in association constant of concanavalin A binding for GDS membranes displaying nano-organized sugars in comparison with homogeneously distributed Man moieties on the GDSs surface or glycolipid membrane analogs.[3] On the other hand the dissociation remained invariant for every type of GDS membranes indicating that the periodic arrays of Man resulted in a new ligand with enhanced reactivity to the lectin concanavalin A.

From this can be concluded that the nanoarrays offer cooperative and multivalent interactions, amplifying the binding response. Thus, these experiments provide a powerful example in which structure determines function, particularly the way different supramolecular assemblies encode biological recognition.

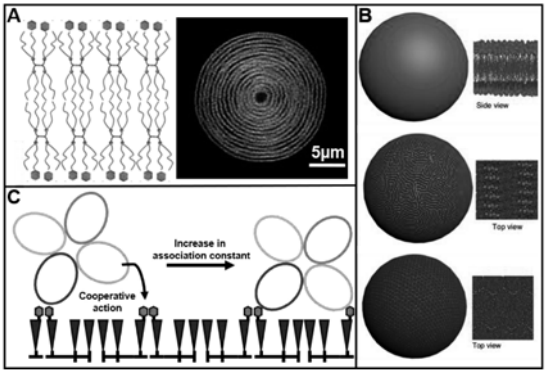

Figure 1. A: Schematic bilayer structure of Janus glycodendrimers and an onion-type glycodendrimersome observed in CSLM. B: Three different observed surface topographies. C: Schematic: Nanoarrays increase the binding of concanavalin A by cooperative interactions.

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Rational design of photochromic analogs of tricyclic drugs

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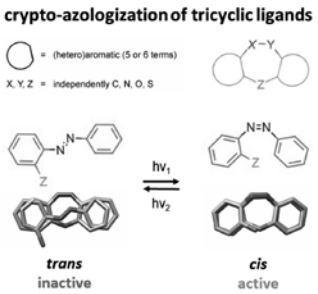
Tricyclic chemical structures are the core of many important drugs targeting all neurotransmitter pathways. These medicines enable effective therapies to treat from peptic ulcer disease to psychiatric disorders. However, when administered systemically they cause serious adverse effects that limit their use. In order to obtain localized and on- demand pharmacological action using light, we have designed photoisomerizable ligands based on azobenzene that mimic the tricyclic chemical structure and display reversibly controlled activity. Several pseudo analogs of the tricyclic antagonist pirenzepine demonstrate that this is an effective strategy in muscarinic acetylcholine receptors, showing stronger inhibition upon illumination both *in vitro* and in cardiac atria *ex vivo*. These photoswitchable "crypto- azologs" of tricyclic drugs might open a general and innovative way to spatiotemporally target their therapeutic action while reducing their systemic toxicity and adverse effects.



ACKNOWLEDGEMENTS

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Super-flexible Biomimetic Vesicles Kill E. coli

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As protocellular research and the study of abiogenesis was driven forward in the last couple decades, researchers had to tackle different obstacles. Essential stability and longevity in conventional cell membrane models were achieved either by utilizing cholesterol in high molar ratios in liposomes, or by entanglement of hydrophobic chains in polymersomes assembled from block copolymers. Both approaches presented their own disadvantages such as a low 2D mobility and storage problems for the former and no biomimetic thickness as well as a low flexibility and 2D mobility for the latter. In this poster, we will present a new class of amphiphiles that are able to self-assemble into super-flexible biomimetic ionic combisomes. We designed and synthesized amphiphilic comb- shaped oligomers where the hydrophobic chains are non-covalently linked to the backbone (Figure 1a). This approach led to new type of vesicles – ionic combisomes – that amalgamate the stability of polymersomes with the biomimetic properties of liposomes while drastically increasing the flexibility (Figure 1b).

These properties of ionic combisomes were achieved by careful selection of monomers and their telomerization into a flexible intrinsic hydrophilic oligomeric backbone to which alkyl chains were attached by ionic interactions. The length of the alkyl chains was selected to represent the biomimetic thickness of the natural cell membrane (5±1 nm).

The flexibility of ionic combisomes can be easily programmed by the grafting density of the alkyl chains along the hydrophilic backbone. These molecules create new possibilities for applications where high dynamics of membrane are necessary. In particular, we are interested in establishing basic means of communication between synthetic cells and natural cells to direct their behavior. Thus, first experiments show that our ionic combisomes instantaneously kill *E. coli*. We observe immediate wrapping of bacteria by vesicles (Figure 1c) and subsequent shape transformation of the bacteria from rod-shaped to spherical. As energy is necessary for *E. coli* to maintain their rod-shape, transformation indicates cellular destruction – cell death. The mechanism of this lethal action on one hand can be compared to that of low-molecular-weight cationic biocides but on the other hand without full disruption of the bacterial cytoplasmic membrane and preserving toxic debris inside of the vesicle.

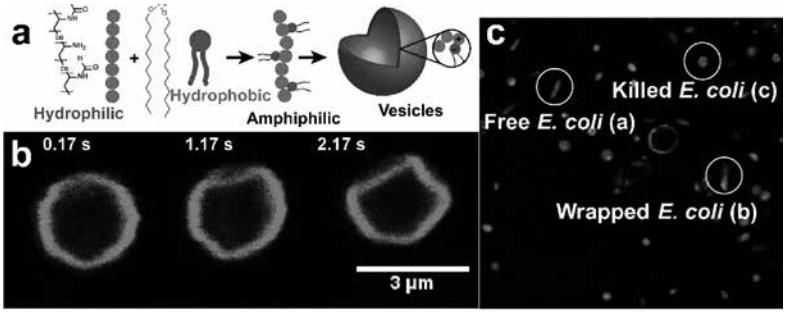

Figure 1 a: Schematic description of the synthesis and self-assembly of ionically linked combisomes. b: CLSM micrographs of strong thermal fluctuations of ionic combisomes at room temperature. c: interaction with *E. coli* showing free, wrapped and killed bacteria.

Polymer Brushes as a Platform to Improve Biocompatibility of the Coatings

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Medical devises and implants are important tools of a health care system that helps diagnose and treat disease and able to improve the quality of patient's life. However, the contact of artificial materials with biological media results in unspecific and irreversible protein adsorption called protein fouling. Fouling leads to the formation of conditioning film that promotes activation of immune response and biofilm formation on implantable medical devices as well as false results of biosensors caused by non-specific binding of non-targeted molecules. Different types of coatings were developed to prevent protein adhesion and improve biocompatibility. The best among them are hydrophilic polymer brushes. However, controlled polymerization of hydrophilic monomers is very challenging and needs to be optimized. We developed a new polymerization protocol based on photoinduced single-electron transfer living radical polymerization (SET-LRP) to synthesize polymer brushes from different hydrophilic monomers including zwitterionic ones.

This polymerization proceeds very fast, requires ppm level of copper catalyst, has high control, and end-group fidelity necessary for the preparation of diblock copolymer and other architectures. Grafted film needs to be uniform with a minimal thickness of 30 nm to provide effective stealth properties. These parameters we controlled using X-ray photoelectron spectroscopy (chemical composition), ellipsometry (thickness), and atomic force microscopy (uniformity). Stealth properties of the coatings were characterized by surface plasmon resonance (SPR). Furthermore, bioreceptors or ligands were introduced utilizing EDC/NHS coupling or catalyst-free strain-promoted alkyne–azide cycloaddition (SPAAC). Oriented immobilization of enzymes was performed using sortase-mediated transpeptidation (sortagging). Remarkably antifouling properties of copolymer brush (N-(2-hydroxypropyl) methacrylamide and carboxybetaine methacrylamide poly(HPMA-co-CBMAA)) and diblock copolymer brush (poly(ethylene glycol) methyl ether methacrylate and azide-functional glycidyl methacrylate) were not affected by immobilization of ligands. The combination of stealth properties with specific recognition allowed the development of surfaces able to improve hemocompatibility of membrane for extracorporeal membrane oxygenator or biosensor capable of detecting thrombin in 10% blood.[1-2] Moreorver, we were able to graft polymer brushes from the surface with complicated geometry, like microporous microparticles and used them to scavenge lipopolysaccharides from blood plasma.[3]

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Unravelling topology-induced shape transformations in dendrimersomes[1]

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The vital functions of cell membranes require their ability to quickly change shape to perform complex tasks such as motion, division, endocytosis, and apoptosis. Membrane curvature in cells is modulated by very complex processes such as changes in lipid composition, the oligomerization of curvature-scaffolding proteins, and the reversible insertion of protein regions that act like wedges in the membrane. But, could a much simpler mechanism support membrane shape transformation? In this poster, I will show how the change of the amphiphile topology (shape) in the bilayer can drive the morphogenesis of cell membrane models. To tackle this, we have designed and synthesized a new type of amphiphiles —Janus dendrimers— that self-assemble into uni- or multilamellar vesicles.[2] Although these molecules do not exist in nature, the formed vesicles closely mimic the thickness, flexibility, and the lateral 2D organization of cell membranes. These properties are precisely encoded in the chemical structure, architecture, and topology of the macromolecular building blocks of the membrane.[3] For these studies, we synthesized Janus dendrimers containing a photo-labile bond that upon UV-irradiation cleave by losing a part of the hydrophilic dendron. This leads to a change from a cylindrical to a wedge- shaped amphiphile. The high mobility of these dendrimers allows for a concentration of the wedge-shaped amphiphiles and the generation of local spontaneous curvature. The concentration of the wedges and their rate of segregation allowed controlling the budding and generation of structures such as tubules, starfish, and high genus vesicles (Fig. 1). Moreover, dendrimersomes were shown to be carriers efficient at loading molecules ranging from hydrophobic small-sized to macromolecular cargos.[4] Hence, this system offers a unique photomediated cargo release and recruitment platform for the design of vesicle based nanocarriers.[5]

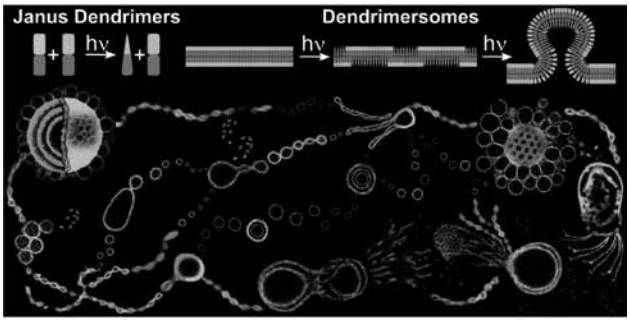

Figure 1: Curvature generation in the bilayer membrane by changing the topology of the amphiphilic building blocks. The topology changes from cylindrical to wedge-shaped by irradiation with a UV-Vis laser leading to a variety of vesicle morphologies such as tubules, starfish, and high genus vesicles.

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Fibrinolytic Coating System to Achieve Adaptive Hemocompatibility of Blood-Contacting Devices

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The major problem of blood-contacting devices is the lack of hemocompatibility of any artificial surfaces that leads to surface-induced activation of coagulation and can result in life-threatening complications. Our strategy to improve hemocompatibility of artificial materials is to design a surface modification that can mimic the way endothelium competes against thrombus formation using positive and negative feedbacks to control thrombus digestion. In the present study, we demonstrate an ultra-thin fibrinolytic coating that prohibits any interaction with blood (stealth properties) and initiation of the coagulation cascade and capable of the rapid disintegration of thrombi in a self-regulated manner in case of their appearance.

The stealth properties were provided by polymer brushes based on poly(*N*-hydroxypropyl methacrylamide-*co*-carboxybetaine methacrylamide) grafted from the surface of the oxygenator membrane. The localized fibrinolysis was achieved by immobilization of tissue plasminogen activator (tPA) on polymer brushes without compromising the antifouling properties of the coating. This enzyme is able to switch between a dormant and an active state depending on the conditions of blood (presence of thrombus). Repellent properties of this coating were evaluated by surface plasmon resonance and scanning electron microscopy. These studies demonstrated that the polymer brushes prevent protein adsorption and the adhesion of platelets and leukocytes. Additionally, no adsorption of Factor XII was detected preventing activation of the contact system by the coating. The prevention of the adsorption of proteins from the contact and complement system guarantee that the inflammation is also inhibited. Importantly, the immobilized tPA was capable of digesting a fibrin-mimic substrate at the physiological concentration in only 15 min. Furthermore, *in vitro* coagulation experiments demonstrated that the fibrinolytic coating delayed thrombus formation, digest the formed thrombi, and completely prevented the adhesion of blood components. These results demonstrate that the combination of passive repellency with adaptive fibrinolysis is a powerful tool to counteract thrombus formation.

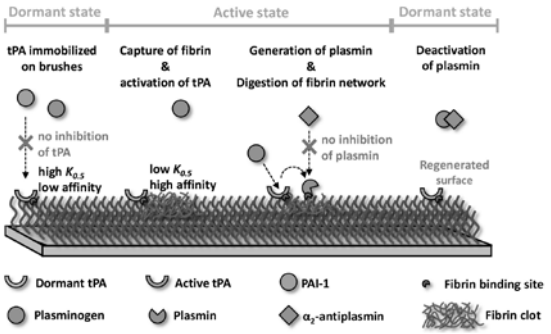

Figure 1: Fibrinolytic coating system developed in the present study. Polymer brushes are decorated with tPA in its dormant state. However, the coating can detect the presence of a fibrin clot by its binding to tPA. This lead to the switching of tPA in its activate state and enables the continuous production of plasmin from circulating plasminogen. The former is produced and released from tPA continuously (amplification) and digests the fibrin clot. Afterwards tPA returns to its dormant state and plasmin is deactivated by its natural inhibitor.

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Laminin impairs mechanosensing by protecting the nucleus from mechanical loading.

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Basement membrane (BM) has the long-standing role as a tissue-supporting element that controls epithelial morphogenesis from early development to later stages of epithelial remodelling during wound healing or cancer metastasis. One of the hallmarks of breast cancer metastasis, for instance, is the breaching of the basement membrane leading to the migration of the invasive front, highlighting the role of BM in protecting tissue integrity. Laminin is the most abundant component of BM and despite its extensive use in numerous cell culture models and its physiological significance in disease progression, very little is known about its influence in cellular mechanoresponses. We found that laminin-111 had a profound effect in dampening cell mechanoresponses in human breast epithelial cell lines. Unlike cells seeded on collagen or fibronectin, cells seeded on laminin exerted lower forces and had compromised mechanosensitivity as shown by reduced YAP nuclear translocation, and smaller focal adhesions (FAs). Blocking integrin β4, a laminin-specific integrin, and structural component of the hemidesmosomes, led to an increase in nuclear YAP ratios, without affecting cell forces and focal adhesion growth. A unique characteristic of integrin β4 is its ability to interact with the keratin network through the cytolinker plectin. Upon blocking integrin β4 the keratin network organization and its dynamic interaction with the actin cytoskeleton were perturbed. By specifically blocking the interaction between integrin β4 and keratins or the cytoskeleton and the nucleus, we show that the laminin-integrin β4- keratin network shields the nucleus from mechanical loading, thereby reducing YAP nuclear localization. Overall, we propose a novel mechanism, by which extracellular matrix composition can influence gene expression, by protecting the nucleus from mechanical loading.

Molecular and physical factors modulating Cajal-Retzius cells migration and distribution in cortical development

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Cajal-Retzius (CR) cells coordinate proper layer cell formation during cortical development. Previously, CR cells migrate tangentially from different sources (cortical hem, pallium subpallium- boundary or septum pallium) and they migrate in certain regions in the entire plexiform-layer I. However, the reason for their differential distribution remains unrevealed. Previous findings point towards a molecular control on CR migration as CXCL12/CXCR4, Sema3E/PlexinD1 or EphB1/B2/B3, but other factors might be involved. Emerging evidence suggests that changes in the mechanical sensing between migrating cells or navigating axons with their environment could play a key role in these processes.

Here, using explant *in vitro* culture we are able to determine relevance of several factors involved on CR cells migration. By changing hydrogel densities in CR cell cultures we were capable to monitor changes of CR cells speed *in vitro*. In addition, we found a new motogenic role of Angiopoietin1 on CR cells migration. Atomic Force Microscopy (AFM), rheometry and Traction Force Microscopy (TFM) techniques, allowed us to infer an effect of physical forces on this mid- stage brain development. AFM revealed stiffness differences on cortex surface whereas TFM showed us the low traction forces exerted by CR cells, which might relay in their particular distribution in the developing pallium.

These findings suggest that physicals factors are involved on CR cells migration. Moreover, the studied molecular factors are postulated also as candidates to modify positioning and migration of CR cells. All together our data point that CR cells are able to respond to chemical as well as mechanical-environmental properties.

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Ultrafast cadherin durotaxis through a wetting transition

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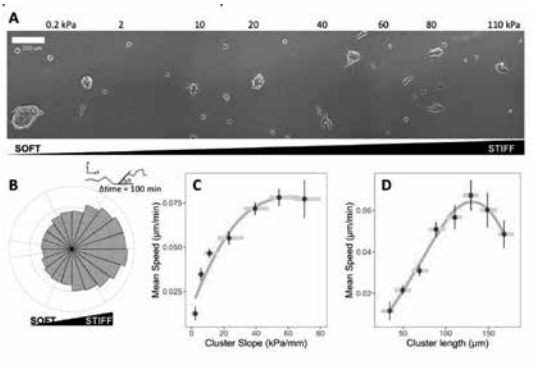
Directed cell migration –the ability of cells to follow environmental cues– is an essential mechanism that it is involved in important tissue processes such as morphogenesis, wound healing and tumor metastasis. This kind of cell migration has been mainly studied when mediated by focal adhesions at the extracellular matrix (ECM) interface. However, important migratory processes during development or metastasis take place in absence of extracellular matrix. These processes are mediated by cadherins, a cell-cell adhesion protein that is essential to maintain tissue integrity, to promote coordination and establish cell polarity. For example, cadherin-mediated migration occurs during drosophila oogenesis, when border cells delaminate from the monolayer follicular epithelium at the anterior end of an egg chamber and directionally migrate as a compact cluster toward the oocyte. In the context of cadherin mediated migration, it is unknown how migratory cells are able to orient themselves to reach their destination.

In this work, we report for the first time that human epidermoid carcinoma cell clusters (A431) systematically migrate from low to high stiffness regions when seeded on a hydrogel coated with oriented E-cadherin molecules, exhibiting a migratory behaviour called durotaxis. We show that E-cadherin-mediated durotaxis increases with cluster size and peaks at a certain local stiffness offset. Interestingly, we found that some A431 cell clusters migrate unexpectedly large distances, presenting a non-gaussian migratory behaviour, a hallmark of ultrafast dynamics. We also found that clusters on E-cadherin-coated substrates display actin-enriched protrusions, while cluster spreading area increases with local stiffness offset. Taken together, these results suggest that durotaxis on E-cadherin-coated substrates is driven by the interplay between traction forces and contractile intercellular stresses in what is called a wetting transition. To date, durotaxis has only been described on ECM coated surfaces such as fibronectin and collagen. In this work we show that durotaxis can also be mediated through E-cadherin, and this might be key for fundamental processes such as development or tumor dissemination.



Clusters of A431 exhibit robust durotaxis. (A) Phase contrast image of A431 clusters on a stiffness gradient hydrogel. Scale on the top indicates stiffness in kPa. (B) Clusters on a stiffness gradient tend to move from the softer to stiffer regions. The rose plot of the angles obtained from cluster trajectories (see inset) indicates a clear trend towards stiffer regions. Using the mean speed in the gradient direction as a proxy for durotaxis (>0 indicates durotaxis, = 0 indicates no durotaxis) we observed that durotaxis response depends on cluster slope (C) and length (D).

Spatial Mapping of the Collagen Distribution in Human and Mouse Tissues by Force Volume Atomic Force Microscopy

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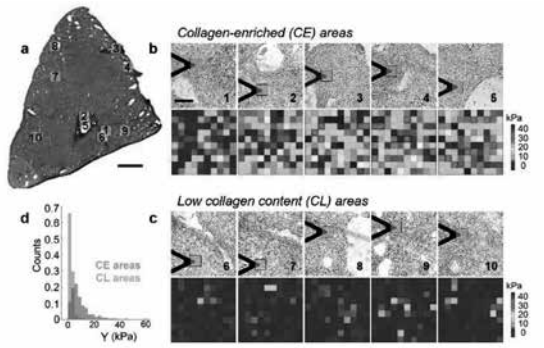
Changes in the elastic properties of living tissues during normal development and in pathological processes are often due to modifications of the collagen component of the extracellular matrix at various length scales. Force volume AFM can precisely capture the mechanical properties of biological samples with force sensitivity and spatial resolution. The integration of AFM data with data of the molecular composition contributes to understanding the interplay between tissue biochemistry, organization and function. The detection of micrometer-size, heterogeneous domains at different elastic moduli in tissue sections by AFM has remained elusive so far, due to the lack of correlations with histological, optical and biochemical assessments. In this work, force volume AFM is used to identify collagen-enriched domains, naturally present in human and mouse tissues, by their elastic modulus. Collagen identification is obtained in a robust way and affordable timescales, through an optimal design of the sample preparation method and AFM parameters for faster scan with micrometer resolution. The choice of a separate reference sample stained for collagen allows correlating elastic modulus with collagen amount and position with high statistical significance. The proposed preparation method ensures safe handling of the tissue sections, guarantees the preservation of their micromechanical characteristics over time and makes it much easier to perform correlation experiments with different biomarkers.



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Mechanical Compartmentalization of the Intestinal Organoid Enables Crypt Folding and Collective Cell Migration

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Intestinal organoids capture essential features of the intestinal epithelium such as folding of the crypt, spatial compartmentalization of different cell types, and cellular movements from crypt to villus-like domains. Each of these processes and their coordination in time and space requires patterned physical forces that are currently unknown. Here we map the three- dimensional cell-ECM and cell-cell forces in mouse intestinal organoids grown on soft hydrogels. We show that these organoids exhibit a non-monotonic stress distribution that defines mechanical and functional compartments. The stem cell compartment pushes the ECM and folds through apical constriction, whereas the transit amplifying zone pulls the ECM and elongates through basal constriction. Tension measurements establish that the transit amplifying zone isolates mechanically the stem cell compartment and the villus-like domain. A 3D vertex model shows that the shape and force distribution of the crypt can be largely explained by cell surface tensions following the measured apical and basal actomyosin density. Finally, we show that cells are pulled out of the crypt along a gradient of increasing tension, rather than pushed by a compressive stress downstream of mitotic pressure as previously assumed. Our study unveils how patterned forces enable folding and collective migration in the intestinal crypt.

Microfluidic device for engineering 3D epithelial monolayers with controlled pressure

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The remarkable feature of the epithelial sheets is to form specialized 3D structures suited to their physiological roles, such as highly branched structures in the lungs, drastic shape changes during embryonic development, or self-organizing organoids. These tissues are distinctive not just in the forms cells assume, but also in function. To achieve this, tissues and the cells in them exhibit coordinated behavior across the spatial and temporal scale. In a sense, 3D epithelia resemble an active material that adapts and changes in response to its biophysical-chemical stimuli like gene expression, morphogen gradients, and lumen pressure. A rheological study of the epithelia would provide unique insight on two fronts. First, to understand the fundamental physical rules of the biology, and second for inspiration of new engineering tools and design principles.

Our study focuses on the tissue response to physical forces, specifically pressure, tension, and curvature. We have fabricated a microfluidic setup to subject epithelial tissues to lumen pressure at different spatial and temporal scales. The epithelial monolayer is grown on a porous surface with circular low adhesion zones. On applying controlled pressure, the monolayer delaminates into a spherical cap (dome). Laplace law for spherical shells allows us to compute tension in the 3D structure with applied pressure and the radius of the dome.

This microfluidic device helps us to characterize the 3D epithelial shape along with the mapping of physical forces. Here, we demonstrate that the device can subject MDCK epithelial cells to a range of lumen pressure at different rates. Drastic reduction in pressure results in tissue collapsing into wrinkles; showing buckling tendency of the tissue under compression. We think that our device enables studying geometrical and biophysical constraints of tissues and unravel emergent phenomena in tissues.

Stromal SMAD3 enhances collective cancer cell migration and invasion led by fibroblasts in 3D culture models

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A hallmark of non-small cell lung cancer (NSCLC) is a fibrotic/desmoplastic stroma rich in activated tumor-associated fibroblasts (TAFs), which are critical regulators of many steps of tumor progression, including migration, invasion and metastasis. NSCLC is currently classified in adenocarcinoma (ADC), squamous cell carcinoma (SCC) and other less frequent histologic subtypes. In addition to a distinct tumor tissue architecture, there is some clinical evidence of different patterns of disseminations in which ADC tumors tend to disseminate earlier than SCC tumors. Intriguingly, we recently found that the important pro-fibrotic TGF- β transcription factor SMAD3 was markedly epigenetically down-regulated in SCC-TAFs compared to ADC-TAFs, which elicited a lower SMAD3 expression and activity that was partially compensated by a larger SMAD2 expression and activity in SCC-TAFs.

Our aim was to assess the impact of altered SMAD3/2 expression in fibroblasts in the migration and invasion of cancer cells. For this purpose, we down-regulated either SMAD3 or SMAD2 by shRNA in primary pulmonary fibroblasts, and used them as SCC-like or ADC-like fibroblast models, respectively. In agreement with our previous observations, shSMAD2 fibroblasts exhibited enhanced SMAD3 expression and signaling as observed in ADC-TAFs, whereas shSMAD3 exhibited enhanced SMAD2 expression and signaling as in SCC-TAFs.

To study collective cell invasion, we for tumor spheroids by mixing lung cancer cells and fibroblasts (1:2) within a 3D gel containing type I collagen mixed with Matrigel. Our preliminary results revealed that, after 48h and in the absence of exogenous TGF-β1, we observed more invasive branches and a higher length in tumor spheroids containing shSMAD2 fibroblasts compared to shSMAD3 fibroblasts. Although these differences were reduced in the presence of TGF- β1, tumor spheroids containing

shSMAD3 fibroblasts remained more invasive. Our preliminary data reveal that SMAD3 in fibroblasts has a robust pro-invasive role in leading collective cancer cell invasion, particularly in basal conditions (i.e. in the absence of exogenous TGF-β1). Our preliminary observations support that the enhanced collective invasion capacity of tumor spheroids containing fibroblasts with enhanced SMAD3 expression (as in ADC-TAFs) may contribute to the early dissemination that is frequently observed in ADC tumors in the clinic.

New system to study single cell mechanoresponse in 3D

As for biochemical signals, the importance of mechanicals signals in processes like the embryogenesis, wound heling or cancer has long been demonstrated opening the field of mechanobiology. Studies on 2D surfaces have allowed researchers to assess cells force transmission patterns but also the cell components required for this force transmission as well as for the transduction of mechanical signals. This way, it also has been shown that the mechanical reaction of a cell to its surrounding is linked to its spreading ability yet this behaviours cannot comprise what is happening *in vivo* as the spreading of a single cell on a 2D surface never happen. In this work, we developed a system enabling us to measure force transmission and transduction in 3D. We then demonstrated the importance of the cell morphology by comparing a 2D system to our 3D system. Force patterns as well as nuclear localizations of the mechanosensitive factor of transcription YAP could not be transposed from the 2D system to our 3D system showing the importance of more complete studies to understand the importance of the 3D cell morphology in mechanotransduction.

Interleukin-1β decreases contractility and attenuates migration and wound-repair ability in primary human pulmonary fibroblasts

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Lungs are soft and elastic to support lung-specific functions. Fibroblasts are increasingly acknowledged as key regulators of tissue mechanics as well as major effector cells in mediating tissue response to damage and biomaterial-host integration in the lungs and other organs. Pro-inflammatory cytokines like interleukin-1β (IL-1β) are released during early responses to tissue damage, and are expected to transiently compromise the mechanical microenvironment. However, the effects of IL-1β on the mechanical performance of fibroblasts remains unclear. To address this gap of knowledge, we treated human pulmonary fibroblasts derived from control donors with IL-1β. Atomic Force Microscopy nanoindentation measurements revealed that IL-1β significantly reduced the stiffness of fibroblasts, concomitantly with a dramatic remodeling of the actomyosin cytoskeleton in which the content of both F-actin and α-SMA was downregulated, and stress fibers were markedly decreased. Likewise, the expression of COL1A1 was reduced, whereas that of COL3A1 was upregulated, eliciting a larger COL3A1/COL1A1 ratio, which has been previously associated with scar-less repair. These changes were functionally associated with reduced fibroblast proliferation, migration and ability to close the gap in an *in vitro* scratch wound assay, which are direct repair-associated functions, thereby supporting that IL-1β may contribute to fibroblast-directed repair through other processes, including enhanced lung distension and innate protection against fibrosis. Our results shed light on the altered mechanobiology of pulmonary fibroblasts by IL-1β, which may be critical during early responses to tissue damage. These findings may also help current research efforts for directing inflammation towards pro-repair rather than pro-fibrosis processes.

Mechanosensitive control of nucleocytoplasmic transport

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Mechanical force controls fundamental cellular processes in health and disease. There is increasing evidence about the physiological relevance of nuclear mechanosensing. For instance, nuclear force increases the import of the transcriptional regulator YAP into the nucleus. However, whether nucleocytoplasmic transport is a mechanosensitive process, and its molecular specificity, remain unknown. Here we show that nuclear forces increase both diffusive and facilitated transport through nucleopores in both directions, and we set the rules for the mechanosensitivity of shuttling proteins. We develop molecular constructs controlling molecular weight and affinity to karyopherins. By tuning the molecular weight we control diffusion, and by increasing the affinity to karyopherins we increase the strength of facilitated transport in both directions. We study the steady state localization and kinetics of these molecular tools in combination with forces. Under force, facilitated import increases more than diffusion, generating a higher nuclear to cytosolic concentration difference. However, a similar increase in facilitated export and diffusion is not able to decrease protein concentrations in the nucleus under force, although shuttling kinetics are faster when force is applied. Furthermore, we show that nucleocytoplasmic transport of key oncogenic transcription regulators such as SNAI1/SNAI2 and TWIST1 is mechanosensitive by this mechanism. Nuclear force provokes the accumulation of both TRs in the nucleus, and ablating force transmission to the nucleus or facilitated transport prevents such accumulation. Using different mutants of TWIST1 we show that the increase of nucleocytoplasmic transport is essential for accumulating TWIST1 in the nucleus. Our results provide a general mechanism by which the nucleus acts as a mechanosensitive organelle via the tight and reversible regulation of passive and facilitated diffusion through nucleopores, controlling the nuclear accumulation of proteins under force.

Imaging based determination of mechanical properties of vesicles and synthetic cells

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Mechanical properties determine the kinetic behavior of giant vesicles. These properties are the result of the molecular composition and the given geometry. However due to the large number of molecules present in the vesicles, it is not possible to derive their mechanical and thermodynamic characteristics from the interactions of their molecular components using theoretical models.

It is always attractive to use methods, where such micromechanical properties can be determined from microscopic observation. Using fluctuation spectroscopy is especially desirable, because the observations do not require any direct manipulation of the sample.

However, in every method promising such simple ways of accessing underlying physical characteristics, there are several assumptions, which are often hard to control.

Fluctuation spectroscopy is special in another aspect. While the problem was first solved more than thirty years ago, reading the literature provides a difficult to understand, often inconsistent framework. In this poster we present a simplified view of the topic and discuss the feasibility of the method based on our experimental data.

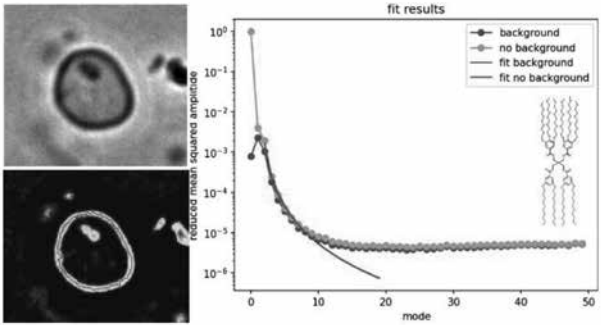

Figure 1: Fluctuation analysis of dendrimersomes. A: the original phase-contrast image recorded using a 20x phase contrast objective (pixel size about 0.22 µm). B: the traced radial profile. On the right: mean fluctuation amplitudes established using 5000 images for the various angular modes. A theoretical fit resulted in a bending modulus of 6.3 ± 1.4 kBT, and a normalized surface tension of about 1.1 ± 0.9). Inset on the right shows the molecular structure of the used dendrimer (IB172-C10).

Dynamic mechanochemical feedback between curved membranes and BAR protein self-organization

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BAR proteins enable essential cellular processes by interacting with and reshaping curved membranes, as encountered in endocytosis, response to osmotic changes, build-up of caveolae or maintenance of cell polarity. The nature of the curvature coupling of BAR proteins on tensed and thin lipid tubes is well understood and involves sensing at low protein concentration, and reshaping at high enough concentration. However, such in-vitro set up does not capture cellular scenarios of curved membrane with low tension and of more versatile shapes and sizes, nor it describes the dynamics of their reshaping process.

We have carried out an in-depth study of the non-equilibrium process of membrane reshaping by the Amphiphysin BAR protein, for a variety of initial membrane templates. To this end, we have developed a new experimental *in vitro* assay where mechanical force applied to a supported lipid bilayer creates physiological-like lipid buds and tubes off the bilayer and subsequent Amphiphysin binding triggers membrane reshaping. We coupled this to a new theoretical and computational framework accounting for membrane mechanics, and the reaction/diffusion kinetics of protein binding. We show that the dynamics of reshaping conforms a very rich process with many intermediate steps, including phase separation between isotropic and nematic phases, and with major reshaping processes occurring at low coverage and low curvature. We extend our *in vitro* findings at the cellular level by showing that Amphiphysin-membrane interaction is triggered upon cell mechanical stretch, enabling potential mechanotransduction mechanisms. Our results characterize and broaden the reshaping processes of BAR proteins on mechanically constrained membranes, demonstrating the interplay between membrane mechanical stimuli and BAR protein response. There is thus ample room to discover novel physical phenomena with potential major biological implications, given the many physiological processes in which BAR proteins are involved.

Linking epithelial geometry to tension and pressure

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Epithelia are thin cellular layers that act as mechanical and biochemical barriers. They are dynamic tissues that present strong intercellular junctions needed to maintain their integrity while growing and regenerating. During embryogenesis, they fold progressively and give rise to highly reproducible 3D geometries that guide the shape and positioning of organs[1].

The way pressure and tension depend on the size of 3D epithelial structures can help us understand how epithelia fold into determined shapes and are able to maintain them even under the continuous remodeling due to cell division[2]. In this project we study the link of epithelial size and shape with luminal pressure and intercellular tension in fluid- filled MDCK 3D monolayer structures.

To guide the 3D folding of an epithelial monolayer, we create micropatterns with different concentration of adhesion proteins on top of 3kPa Polydimethylsiloxane (PDMS) gels[3]. We then seed cells expressing a cell membrane marker that allows tracking of the epithelium's curvature. The difference in adhesion between regions permits the delamination of cells to form fluid-filled pressurized structures of different sizes and shapes (spherical caps, rectangles, ellipses...). Using Traction Force Microscopy, we can measure the forces exerted by the cells on the substrate, as well as luminal pressure inside of the 3D structures[4], and relate them to cell tension and epithelial curvature.

Our findings indicate that luminal pressure decreases with the footprint radius in spherical cap monolayers, but, surprisingly, this does not represent an increase in tissue tension. In rectangular structures, we see an asymmetric distribution of cell tractions, with cells exerting more force on the longer sides of the curved monolayer. These results give us an insight into how size and shape of fluid-filled lumens can affect growth and mechanical properties of the surrounding epithelia.

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Low-cost device to measure maximal inspiratory (MIP) and expiratory (MEP) pressures

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Measurement of MIP and MEP help in diagnosing and following-up respiratory muscle weakness in patients with neuromuscular, cardiac and respiratory diseases. Commercial devices including MIP-MEP are expensive being unaffordable in low/middle-income countries (LMICs). Our aim is to design and test a high-performance hand-held low-cost device for measuring MIP-MEP METHODS: The device consisted of pressure transducer (SSCDRNN160MDAA5, Honeywell), an Arduino microcontroller, and an LCD screen. All the external components of the portable device were designed and implemented in PLA by using an FDM 3D printer. To validate the usability and performance of the implemented prototype, the device was compared (in a range of ±80cmH2O) with a laboratory reference setting.

RESULTS: The total retail cost of the devices was below 80€. The mean difference with the reference was 0.13cmH2O with narrow limits of agreement.

CONCLUSIONS: Given the open source hardware availability, this performant low-cost device can be readily assembled by users in LMICs, expanding the accessibility of this medical device.

Low-cost open-source pressure support ventilator for patients with respiratory failure

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Most hospitals and patients in low/middle-income countries (LMICs) cannot afford the current pricing schedules of commercial mechanical ventilators. This has resulted in a marked restriction of their availability, and leaft patients with respiratory failure with restricted access to this lifesaving treatment. Our aim is to design low-cost and easy-to-build non-invasive bilevel pressure ventilator that should reduce the serious shortage of ventilators in LMICs.

METHODS: The ventilator is built using off-the- shelf components available via e-commerce, and is based on a high-pressure blower, two pressure transducers and an Arduino Nano controller with a digital display (total retail cost <75 US$). The designed ventilator allows independent setting of inspiratory and expiratory pressures up to 20 and 10 cmH2O, respectively, selectable cycling threshold and a back-up rescue frequency (assisted-controlled ventilation mode). All construction details are provided following an open source hardware and code approach for free replication.

RESULTS: The implemented non-invasive ventilator presented equivalent performance to high-quality commercial devices when tested in healthy volunteers.

IRSp53 orchestrates local actin polymerization to remodel plasma membrane upon stretch

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In a range of physiological processes from extravasation to endocytosis, cells are constantly submitted to shape changes, which eventually involve plasma membrane remodelling and adaptation. Potentially, this remodelling can be harnessed by cells to detect and respond to shape changes, enabling mechanosensing mechanisms. However, how such process occurs is still largely unknown.

We have engineered a system that allows us to induce plasma membrane reshaping through controlled stretch deformations while monitoring cell response by live imaging. We have observed that under de-stretch cells passively respond by forming membrane evaginations. Such folds resorb actively in a process regulated by the I-BAR protein IRSp53. Our results suggest that this protein orchestrates a node of actin polymerization that flattens the structure allowing the cell to come back to the steady state. Accordingly, IRSp53- directed actin polymerization would be the first step in the mechanosensitive cascade that mediates cell response to stretch.

Cyclic stretching mesenchymal stromal cells cultured on lung hydrogels

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Our aim is to develop an *in vitro* model where cells are subjected to physiological mechanical stimuli when cultured in lung-ECM derived hydrogels. METHODS: Rat primary lung resident MSCs were cultured on top of 3D porcine lung extracellular matrix (ECM) hydrogels with a stiffness of 0.7kPa. Hydrogels were attached to a custom-made lung-on-a-chip device with a PDMS flexible membrane allowing cyclic stretch (control cells were attached to the PDMS by using standard collagen I functionalization of the membrane). Cyclic stretch of 10% at breathing frequency (0.2Hz) was applied to the cultured cells. After 5h of stimulus, actin and paxillin were stained to measure the length of focal adhesions of the cells.

RESULTS: Interestingly, cyclic stretch showed to reduce the length of focal adhesions in the cells cultured in standard conditions (2.5±0.2μm vs 1.8±0.1μm), but to increase them when cultured in lung-ECM hydrogels (1.4±0.1μm vs 1.7±0.1μm).

DISCUSSION: The data indicate that the effect that has culturing lung MSCs under cyclic stretch is dependent on how the mechanical stimuli are transmitted to the cells. Thus, lung ECM-derived hydrogels could be a useful scaffold for the development of novel *in vitro* models based on lung-on-a-chip devices.

Diffusioosmotic and convective flows induced by a non-electrolyte concentration gradient

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Glucose is an important energy source in our bodies, and its consumption results in gradients over lengthscales ranging from the sub-cellular to entire organs. Concentration gradients can drive material transport through both diffusioosmosis and convection. Convection arises because concentration gradients are mass density gradients. Diffusioosmosis is fluid flow induced by the interaction between a solute and a solid surface. A concentration gradient parallel to a surface creates an osmotic pressure gradient near the surface, resulting in flow. Diffusioosmosis is well understood for electrolyte solutes, but is more poorly characterised for non-electrolytes such as glucose. We measure fluid flow in glucose gradients formed in a millimetre-long thin channel, and find that increasing the gradient causes a crossover from diffusioosmosis-dominated to convection-dominated flow. We cannot explain this with established theories of these phenomena which predict that both scale linearly. In our system, the convection speed is linear in the gradient, but the diffusioosmotic speed has a much weaker concentration dependence, and is large even for dilute solutions.

We develop existing models and show that a strong surface- solute interaction, a heterogeneous surface and accounting for a concentration- dependent solution viscosity can explain our data. This demonstrates how sensitive non-electrolyte diffusioosmosis is to surface and solution properties and to surface- solute interactions. A comprehensive understanding of this sensitivity is required to understand transport in biological systems on lengthscales from micrometres to millimetres where surfaces are invariably complex and heterogeneous.

Smart skeletons for 3D printed living biobots

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The development of nano and microrobots based on biomimetic designs aim to create advanced functional robotic platforms able to both actuate and present some adaptive mechanisms like their biological counterparts [1]. Some of the desired unique from this living entities include self-healing, energy efficiency, power-to-weight ratio, adaptability, or bio-sensing capabilities [2]. Although most of the designs resemble the well-known structures in nature (i.e. medusoid that mimics a jelly-fish motion dynamic [3]), simpler structures have served to stablish key design rules for efficient bio-robotic platforms.

Some examples are a cantilever structure where cardiac cells where immobilized [4] or a system based on two legs joined by a beam where a 3D skeletal-muscle cell construct is assembled [5]. In our case, we developed a skeleton-muscle based bio-robot with an integrated compliant skeleton based on a 3D-printed spring serpentine [6]. Such configuration not only provided mechanical integrity to the biobot system, but also allowed an on-demand bending and a mechanical self-stimulation in absence of any external electrical input. Corresponding finite element analysis were done to both find (i) the optimal geometrical stiffness to achieve the desired asymmetry/ buckling effect for efficient motion and (ii) the mechanical self-stimulation for an enhanced output force, demonstrating also two types of motion mechanisms for the same biobot configuration.

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Cell-substrate adhesion drives intestinal epithelial cell organization

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Understanding epithelial cell organization is of fundamental importance for several physiological and pathological processes such as tissue homeostasis, regeneration and cancer[1,2]. For example, intestinal cells self-organize into 3-dimensional (3D) organoids that recapitulate the *in vivo* structural and functional characteristics when embedded in a 3D cell-derived protein mixture[3]. However, these very same cells self-organize into 2-dimensional (2D) intestinal epithelial monolayers that recapitulate the *in vivo*-like cell type composition and organization when seeded on thin layers of the same cell-derived protein mixture[4,5]. In general, changes in epithelial cell organization are characterized by a cross-talk between cell-substrate and cell-cell interactions[6], but the role of dimensionality, protein composition and concentration in their complex self-organization process is not fully understood.

Here, we show that intestinal epithelial cells self-organize in 2D-monolayers or 3D-tubular networks depending on Matrigel protein concentration when the dimensionality is fixed. We found out that intestinal organoids-derived single cells spontaneously self-assemble to form tubular networks with inner apical polarization when seeded on thin protein layers above a threshold concentration. These networks are similar to soap foams or de-wetted collagen networks[7] and have well defined topological and metrical properties, distributed according to a Voronoi tessellation. The network becomes spontaneously ordered at large length scales and the formation process occurs at nearly the same time across a large distance. In contrast, on low Matrigel concentration, intestinal organoids-derived single cells form 2D intestinal epithelial monolayers. Interestingly, we found a different stem cell dynamic during the formation of each self-organized patterns. On low Matrigel concentration, stem cells performed a confined random walk forming to a 2D-monolayer. In contrast, on higher Matrigel concentration, stem cells behaved as non-confined random walkers to form the 3D-tubular networks. By reducing the proportion of stem cells in the culture, the formation of 3D-tubular networks was impaired. Instead, primary cells formed aggregates when seeded above the transition protein concentration, similar to two

other epithelial cell types (Caco-2 and MDCK cells).

Our experiments illustrate how Matrigel concentration regulates intestinal epithelial cell organization as a function of cell-substrate adhesion, and show that primary intestinal epithelial cells self-organize in structures with well-defined sizes and shapes independently of dimensionality or external signaling gradients. Also, we show that the amount of stem cells in the culture regulates the geometry of those self-organized structures. Our work could yield insights about the roles of stem cells and protein concentration in tissue morphogenesis and their influence in the *in vivo* tissue morphological features such as the dimension of the crypts.

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The Capacity of RGD and DWIVA Peptidic Biointerface to Transdifferenciate C2C12 Myoblasts into Osteogenic Lineage

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Mimicking the extracellular matrix is particularly important to enhance cell-material interactions. To this end, surface modifications with osteoconductive and osteoinductive properties are essential to engineer bone tissue. In this regard, biomaterial's functionalization with integrin- binding molecules, such as the RGD motif, has been a main goal. Moreover, growth factors (GFs), like bone morphogenetic 2 (BMP-2), are also of relevance to recreate the complexity of the healing microenvironment. However, the use of BMP-2 entails some clinical risks, such as ectopic bone formation and inflammation [1]. To overcome these drawbacks, the identification of BMP-2 derived sequences and their combination with RGD-based ligands to simultaneously induce integrin and GF signaling is a promising alternative.

In the present work, a multifunctional peptidic biointerface containing the RGD cell adhesive motif together with the DWIVA peptide (derived from the wrist epitope of BMP-2), was used to study the synergistic promotion of integrin/GF signaling on C2C12 myoblasts. This biomolecule was modified with L-3,4-dihydroxyphenylalanine (DOPA) as anchoring unit to ensure its binding to model glass substrates [2].

C2C12 myoblasts are known to fuse and differentiate, forming myotubes under standard culturing conditions. However, when they are exposed to BMP-2, their myogenic differentiation is inhibited and they start to transdifferentiate into the osteogenic lineage [3]. Based on these findings, C2C12 cells were cultured on glass substrates modified with the RGD-DWIVA peptide and cell adhesion, their transdifferentiation capacity and BMP-2 dependent signaling were studied. To ensure the potential of the peptidic biointerface, different controls were also tested: RGD (with scrambled DWIVA), DWIVA (with scrambled RGD), RGD with soluble DWIVA, RGD-DWIVA with integrin blocking and a mixture of RGD and DWIVA (without controlling the spacing). Cell adhesion results showed that the use of RGD-DWIVA within the biomimetic interface resulted in a significant increase in both cell number and cell projected area in comparison with the rest of the conditions, indicating that integrin and BMPRs may synergistically interact to improve C2C12 adhesion. After 6

days in culture, myosin area quantification demonstrated the suppression of myotube formation only in the RGD-DWIVA condition. Finally, western blot results proved that the BMP signaling pathway was Smad-independent as shown by the high expression of p38 [4].

Thus, our strategy showed for the first time a synergistic cooperation between RGD and DWIVA motifs in promoting C2C12 adhesion, transdifferentiation and BMP-dependent signaling through Smad-independent pathway and paves the way for its application to develop GF-free biomaterials with osteogenic potential.

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Microfluidic System to Mimic the Bone Healing Microenvironment and Study the Role of Calcium in Endothelial Progenitor Cell Recruitment

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Over the last decades, most of the strategies to improve the vascularization of biomaterials based on calcium phosphates (CaPs) are based on the incorporation of well-known proangiogenic agents, such as growth factors, despite their high cost and the complexity of delivering safe and effective doses [1]. A promising alternative is the use of inorganic elements that naturally occur within the body, namely metallic ions [2]. It has been shown that dissolution products of CaPs are able to induce vascularization [3,4], although the particular mechanism by which calcium stimulates this process is not very well understood, mainly due to the lack of suitable *in vitro* and *in vivo* models.

In this work, we present a microphysiological system (MPS) to study the role of calcium in neovascularization. The bone-healing microenvironment was mimicked by 3D-culturing bone marrow rat mesenchymal stem cells (BM-rMSC) and rat endothelial progenitor cells (rEPC)either in mono or co-culture conditions. Migration assays were performed in our proposed system, showing that calcium-enriched media (10 mM) is only able to elicit a strong migratory response on endothelial progenitor cells when they are in co-culture conditions. We also show that calcium exerts a potent chemotactic effect on BM-rMSC and induces an increase in the osteopontin (OPN) secretion, a protein involved in chemotaxis and immune regulation [5]. Therefore, we propose a novel mechanism by which calcium can stimulate endothelial progenitor cell recruitment and subsequent vascularization and open up new possibilities to test calcium-releasing biomaterials using MPS.

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Studying Wilms' Tumor 1 (WT1) function in human kidney development and disease using human pluripotent stem cells-derived organoids and genome editing

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The Wilms' tumor 1 (WT1) gene was first characterized in the context of the childhood kidney cancer, Wilms' tumor, and was rapidly shown to play a role in the development of several organs. In disease, WT1 can be inactivated by mutations, acting as a tumor suppressor gene as in Wilms' tumors. So far, most of what is known about WT1 function in development and disease comes from mice models. Mice have many advantages as model organisms, but also shortcuts. For instance, the function of a protein and its regulation described can differ between mice and humans. Therefore, it is necessary to have models closer to humans in order to properly understand kidney development and disease.

Nowadays it is possible to have "human-like" models thanks to human pluripotent stem cells (hPSCs). These cells can be differentiated towards any kidney like-cells, recapitulating early events of human development thanks to the establishment of fast and efficient organoid differentiation protocols. In addition, they are an amenable source for genome editing, allowing us to use tools like CRISPR-Cas9 technology to study the role of WT1 in human kidney development and disease.

Therefore, in the lab we have generated WT1 knock-out hPSC clones, heterozygote and homozygote, to study the impact of the lack of WT1 in early kidney development. Moreover, we have also generated WT1 knock-in clones carrying a hotspot mutation, R394W, which has been described in patients with Wilms' tumors. These clones will give us insight on the effect of a specific patient-related WT1 mutation in kidney development. In order to characterize the resulting phenotypes when these cell lines are differentiated towards kidney organoids, we have analyzed the mRNA and protein expression levels of renal development markers during renal vesicle stage and nephron structure stage. Preliminary data show that both the stable WT1 knock-out and knock-in mutations lead to an impairment of renal development.

In parallel, we have generated and characterized a WT1-GFP reporter cell line, which will enable us to easily isolate the different WT1+ cells that occur during kidney development, ranging from cells of the metanephric mesenchyme to podocytes, and further investigate the molecular mechanisms in which WT1 is involved depending on the cellular context by means of ChIP-Seq and RNA-Seq.

In situ Metabolomics in 3D cell cultures in Organ-On-Chips by hyperpolarization-enhanced Magnetic Resonance

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Organ-on-a-Chip (OoC) devices have emerged as reliable drug testing platforms able to emulate a physiologically relevant cellular microenvironment. Patient-derived cells can be cultured in microfluidic chambers, stimulated with physical and biochemical cues, and ultimately challenged with drug treatments, which offers promising opportunities for the development of precision medicine. As OoC technology advances, it has become important to provide OoC with suitable readouts and make them compatible with current analytical techniques.

Hyperpolarisation-enhanced Magnetic Resonance (MR) techniques, such as Dynamic Nuclear Polarization (DNP), may enhance the sensitivity of MR by 10.000 times and allow us to perform in-situ metabolomic analysis in OoC. DNP-MR combined with OoC provides an unprecedented access to trace labelled metabolites and their reaction kinetics. Therefore, patient-derived tissues can be generated in OoC devices while metabolic processes are non-invasively interrogated using DNP-MR.

We aim to develop a drug screening platform that merges both technologies: OoC and DNP-MR. To overcome this challenge, we have developed a microfluidic device composed of 1) a cylindrical chamber to accommodate a scaffold with cells, 2) an embedded transmit/receive radiofrequency coil surrounding the scaffold to detect the MR signal, 3) a passive membrane pump to inject the labelled sample into the cell chamber, and 4) microfluidic channels to supply the cells with continuous culture media. We have fabricated the device using polydimethylsiloxane (PDMS) soft lithography and have integrated a saddle coil of copper wire inside the PDMS. Cylindrical macro- porous carboxymethyl cellulose (CMC) scaffolds were produced using a cryogelation technique (cryogels); their cylindrical shape maximizes the MR detection volume of the coil.

Preliminary data indicates that the homogeneity of the magnetic field, which is critical for MR data quality, can be precisely shimmed when using the cryogel. A rapid injection of labelled metabolites was resolved using a microfluidic channel connected to a passive membrane pump, thus enabling a reproducible temporal

injection of the tested substrate. An additional microfluidic channel allows the continuous supply of nutrients and oxygen to the cells for long-term experiments. The non- invasiveness of DNP-MR offers the possibility of longitudinal studies, which are not possible with terminal assays. The combination of DNP-MR with personalized OoC models will advance the development of functional person-specific drug testing systems; we expect the incorporation of analytical techniques in-situ to accelerate the implementation of personalized treatments.

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Microengineered Villus-Like PEGDA Hydrogels Under Spatio-Biochemical Gradients For Primary Intestinal Epithelium *In Vitro* Model

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The intestinal epithelium is formed by villi and crypts. Intestinal stem cells (ISCs) located at the crypt base divide giving rise to proliferative cells that migrate up along the villi while differentiating, ultimately dying at the tips of the villi. This homeostasis is tightly controlled by biomolecular gradients of EGF, Wnt and BMP signaling pathways along the crypt-villus axis1. Intestinal organoids, despite including many physiologically relevant features, are not valid cultures when access to the lumen is required. Here we present a culture platform that overcomes this limitation while comprising all key features of the intestinal epithelium: 3D architecture, proliferative and differentiated cell domains, and gradients of ISCs niche biomolecules.

Employing a simple photolithographic technique2, we fabricated poly(ethylene) glycol diacrylate (PEGDA) 3D villus-like scaffolds. We developed *in silico* models to simulate gradients of ISCs niche biomolecules, we created them through the hydrogels by free diffusion and we characterized them by Light-sheet fluorescence microscopy.

Organoid-derived intestinal epithelial cells covered the whole scaffold surface. The gradients profile and composition, constant over time, impacted on cell behavior by modifying the proportion and positioning of the different intestinal epithelial cell types along the vertical axis of our scaffold, faithfully recreating *in vivo* cell compartmentalization.

We have developed an apically accessible and 3D *in vitro* intestinal epithelial model, which bears biomolecular ISC niche gradients and all relevant epithelial cell types. Therefore, we believe our model can be employed in many applications, particularly in the study of intestinal epithelium biology in physiological and pathological conditions.

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Towards a non-invasive biomarker of myotonic dystrophy 1 using NMR-based assays of muscle cells

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Myotonic dystrophy 1 (DM1) is a life-threatening and chronically debilitating disease – the most common cause of muscular dystrophy in adults -, afflicting over 93.000 Europeans. There are two main obstacles for day-to-day patient management and discovery of efficient anti-DM1 drugs: 1) the fact that to assess muscle function of DM1 patients undergo muscle biopsies, with their associated pain and risks; and 2) the long delay between treatment and evaluation of its efficacy. Thus, efforts to find reliable biomarkers of DM1 and early treatment response are crucial.

In order to identify a metabolic biomarker of DM1, patient myoblasts were seeded with diferentiation medium. A week later myoblasts completed the differentiation into myotubes and cells and supernatant were collected. The supernatant and the cell lysate were studied by NMR.

The ^{1}H-NMR spectra ofmyoblast lysatesdisplayed severalintracellularmetabolites of the glucose metabolic pathway, such as glutamine, glucose, lactate, valine, leucine or isoleucine (Fig. 1). The main difference between healthy and DM1 myotubes was a dramatic 10- fold increase of the lactate peak in the DM1 sample. Our current hypothesis, based on this NMR results, is that pyruvate generated from glucose metabolism via glycolysis in DM1 cells is preferentially converted to lactate by lactate dehydrogenase (LDH) as opposed to entering the tricarboxylic acid cycle.

We will continue exploring this hypothesis and test it using the “hyperpolarisation” technique dynamic nuclear polarisation (DNP), which increases the sensitivity of the NMR signal by 50,000-fold and enables real- time, in situ NMR data acquisitions for quantitative assessment of living cells metabolism. This hyper-intense signal will be used to monitor metabolic processes such as enzymatic reactions and transient metabolic reaction intermediates in cell cultures in situ and in mouse models *in vivo*.

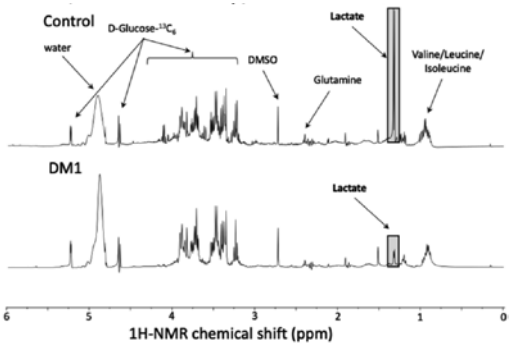

Figure 1: ^{1}H-NMR spectra of intracellular polar metabolites of DM1 myotubes (top) and healthy myotubes (bottom). The concentration of lactate is strikingly increased in DM1 condition. These data were acquired using CPMG pulse sequence with presaturation: 16 scans, 1.4 s acquisition time, 5 s relaxation delay, 5734 Hz spectral bandwidth, 298 K, D_2O solvent, 500.13 MHz spectrometer frequency.

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Development of a breast cancer model based on elastin-like recombinamers for drug discovery

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The tumor microenviroment (TM) is a three-dimensional (3D) system formed by cells, non-cellular components and extracellular matrix (ECM), being all important in tumor progression and metastasis. Current two-dimensional (2D) *in vitro* models used in drug screening cannot fully mimic TM complexity, and more predictive 3D *in vitro* models are needed. Among them, cancer spheroids (cS) can reproduce many tumor features, like the 3D environment, drug resistance and ECM deposition. Hydrogels (HGs) can be used as platforms for cS production. Elastin-like recombinamers (ELR) are elastin-inspired polymers. ELR can self-assembles forming gels above certain temperatures, and can be cross-linked by click chemistry.1 Their tunable properties and biocompatibility make them promising materials for cancer research. The aim of this work is to evaluate whether ELR HGs are suitable for developing breast cancer cS models for drug screening.

HGs were fabricated by mixing i) MMP-sensitive ELR with cyclooctine groups (C-ELR) and ii) RGD- carrying ELR with azide groups (N-ELR), both dissolved in cell media. Breast cancer (MCF-7, MDA- MB-231) or non-tumor breast (MCF10A) cells were dispersed in C-ELR, mixed with N-ELR, and incubated at 4oC for 8 min and 37oC for 15 min. Then, cell media was added on top. Cell proliferation and viability were determined by alamarBlue and calcein AM/propidium iodide. Cell distribution in HGs was studied with Phalloidin-Alexa488/DAPI staining. ECM deposition was evaluated by immunofluorescence. For drug resistance experiments, HGs were incubated with doxorubicin (Dox) for 48h. Cells cultured on culture plates were used as control. IC50s were measured with alamarBlue.

MCF-7, MCF10A and MDA-MB-231 were successfully encapsulated in the HGs, being homogeneously distributed. HGs were biocompatible, and MCF-7 and MCF10A were able to form cS in ELR HGs, whereas MDA-MB-231 formed cell networks within the HGs (Fig. 1). All cell types deposited ECM proteins (collagen IV and fibronectin) after 7d. Also, higher resistance to Dox was observed in cells cultured in HGs compared with 2D cultures. These findings suggest that ELR HGs are a promising material for developing *in vitro* breast cancer models for drug screening.

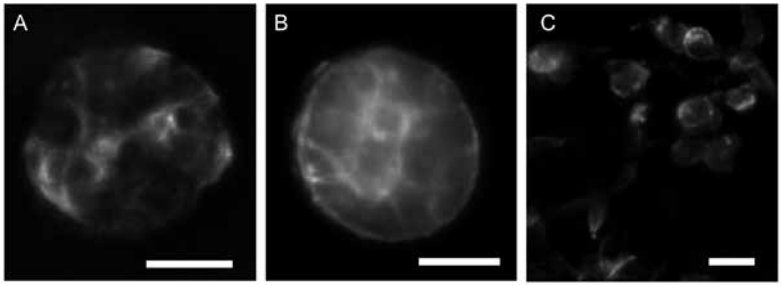

Figure 1. MCF10A (A), MCF-7 (B) and MDA-MB-231 (C) encapsulation in ELR HGs. Cell morphology after 7d in ELR HGs, in blue, nuclei; and green, actin. Scale bar 25 µm.

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Generation of human and pig kidney decellularized extracellular matrix: towards the fabrication of kidney-specific bio-inks for 3D Bioprinting applications

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Decellularized extracellular matrix (dECM) from tissues and organs constitutes a type of biomaterial that contains tissue-specific biochemical cues and the right proportion of ECM proteins from the original native tissue, recapitulating organ-specific microenvironments for cellular growth and function. Already several laboratories including us have shown that the use of dECM scaffolds for recellularization can provide a proper structural and functional background for instructing human pluripotent stem cells differentiation towards the generation of human tissue analogues. Remarkably, through the exposure of hPSCs to three-dimensional culture conditions and differentiation signals, the field has started to define protocols for the generation of organ-like structures containing multiple cell types, so called, organoids. Although valuable, organoid models still lack proper organ-like vascularization and maturation. In this regard, dECMs offer a suitable biomaterial to generate biocompatible hydrogels, known as bionks, which can mimic tissue-specific microenvironments for bioengineering applications including 3D bioprinting. Our laboratory has recently published a methodology for the successful differentiation of hPSCs into kidney organoids that transcriptomically matched second trimester human gestational kidney.

Based on this knowledge, we hypothesize that the combination of hPSCs with kidney derived dECM bioinks would better recapitulate the kidney specific ECM microenvironment allowing for the derivation of organoids with enhanced cellular complexity and function. Here, we have defined the suitable decellularization conditions for fabricating pig and human kidney dECMs using an immersion decellularization protocol. After decellularization, pig and human dECMs were characterized for the absence of cellular material and DNA, and the preservation of the major kidney ECM proteins as well as kidney architecture. In addition, pig

and human dECMs were assayed for their bioactivity and capability to induce blood vessel growth using the chick chorioallantoic membrane (CAM) assay. Next, through the enzymatic digestion of the dECMs, we have established a procedure to fabricate pig and human kidney dECM hydrogels. These have been mixed with different proportions of well-known natural biomaterials (i.e., gelatin, fibrinogen) to generate kidney-specific bioinks with proper rheological properties and biocompatibility for 3D bioprinting. Overall, this work paves the way for the generation of biocompatible hydrogels for further applications in tissue cell culture (i.e., organoids) and tissue engineering (i.e., renal bio- fabrication).

Erasing metabolic alterations in proximal tubular cells under hyperglycaemic condition using inducible CRISPR/Cas9 PGC1a hESC-derived 3D kidney organoids

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There are about 60 million people with diabetes in the European Region. Among all the complications, Diabetic Nephropathy (DN) is the leading cause of end stage renal disease and it can explain most excess mortality associated with diabetes.

From all the kidney cells type, proximal tubular renal cells (PTC) represent one of the most vulnerable cell types in DN due to its high-energy demand, rendering it susceptible to cellular injury. It has been difficult to induce PT cell differentiation in cultured kidney organoids, however our group has demonstrated that kidney organoids under cell culture medium favouring oxidative phosphorylation conditions enhanced tubule differentiation. Due to the increasing evidences which suggest that the metabolic state of a cell contributes to disease development we hypothesize that diabetic nephropathy (DN) is promoted by the metabolic alterations (hyperglycaemia) occurring during kidney development mainly in PTCs. We propose that these metabolic alterations can be erased modifying the intracellular metabolic profile of the kidney cells in a mitochondrial metabolism-dependent manner.

First, to check if hyperglycaemia has an effect in the development of PTC in kidney organoids, we cultured them under oscillatory glucose levels versus constant normal glucose for 7 days at day 16 of differentiation. Preliminary results showed that kidney organoids treated under oscillatory glucose had lower expression of PTC makers and lower number of PTCs than control kidney organoids analysed by RT-PCR and flow cytometry analysis, respectively. In order, to characterize PTCs from oscillatory glucose versus control kidney organoids, we isolated and cultured them in renal epithelial cell growth medium (normal glucose concentration) for a month. PTC isolated from diabetogenic kidney organoids showed higher oxygen consumption rate than PTC from control group. No changes were found in mitochondria copy number neither in oxidative phosphorylation (OXPHOS) complexes expression by western blot. However, we found lower expression of the mitochondrial master regulator PGC1a in PTC from diabetogenic organoids by RT-PCR and immunofluorescence.

Into the light of the results and knowing that cultured organoid in an OX-PHOS promoting media arise higher number of PTC in kidney organoids, we generated an inducible CRISPR/Cas9 engineered line for PGC1α. Overexpression of PGC1a during kidney organoid development showed higher expression of tubular markers such as

SLC3A1 and AQ1 analysed by RT-PCR however we did not find differences at PTCs number by flow cytometry. To study deeply the effect of PGC1a under hyperglycaemic condition, we cultured PGC1a inducible organoids under oscillatory glucose levels versus constant normal glucose for 7 days. PTC from PGC1a inducible CRISPR/Cas9 kidney organoids cultured under diabetogenic condition rescued the expression of PGC1a and the oxygen consumption rate of the PTC.

In conclusion, this preliminary work showed: 1) metabolic programming plays a role in the development of PTC from kidney organoids, in the context of hyperglycaemia 2) metabolic memory can be demonstrated using kidney organoids 3) the mitochondrial master regulator, PGC1a is downregulated in PTC coming from kidney organoids under diabetogenic condition 4) PGC1a inducible CRISPR/Cas9 kidney organoids could have a protective role under diabetogenic condition rescuing the PGC1a expression and the oxygen consumption rate of the PTC. The information gained from this DN modelling will offer improved insight into the mechanism underlying the metabolic state of PTC and the development of the disease.

PrP^C regulates alternative splicing of tau by modulating GSK3β activity

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Tau is a microtubule-associated protein responsible for microtubules (MT) stabilization and, in humans, it is encoded by the MAPT gene which is alternatively spliced to produce six protein isoforms. Exons 2, 3 and 10 are susceptible to alternative splicing that generate the isoforms different in the number of inserts of 29 amino acids at the N-terminal part (exon 2 and 3) and the number of MT-binding repeats, 3 or 4, at the C-terminal part (exon 10) [1]. In this sense, the inclusion of exon 10 yields the tau4R isoforms while its exclusion forms tau3R isoforms that have less MT binding capability and are more prone to phosphorylation [2]. In addition, tau4R has a role in neuronal differentiation and tau3R is predominant during embryonic development or specific neuronal types [1].

In the adult human brain equal levels of tau3R and tau4R are expressed. However, in several neurodegenerative diseases named tauopathies an imbalance on the tau3R/4R ratio occurs in parallel to an intracellular accumulation of hyperphosphorylated tau protein. In this sense, an alteration on tau isoforms balance or tau phosphorylation levels induces the self-aggregation process which leads the formation of neurofibrillary tangles (NFTs). These NFTs bring into MT instability causing an impaired axonal transport and synaptic dysfunction as occurs in Alzheimer's disease (AD) [3]. In the complex control of exon 10 alternative splicing and tau phosphorylation are implicated numerous factors including different miRNAs and several kinases, where GSK-3β represents a key element in both processes [4].

In the other hand, cellular prion protein (PrP^C) is a glycoprotein highly expressed in central nervous system that has different physiological functions in neuroprotection and neuronal differentiation [5]. Several studies suggested roles of PrP^C in promoting neuronal differentiation, probably through its capability on GSK-3β inhibition [6]. Recently, a relationship between PrP^C and tau has been reported in AD [7].

For this reason, we aim to analyze the role of PrP^C and the implication of the GSK-3β in the regulation of tau exon 10 alternative splicing. Using several human samples, mice models and neuronal primary cultures, our results showed that PrP^C modulates the expression of tau isoforms tau3R and tau4R, through the inhibition of GSK-3β activity.

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Development of a drug screening model using 3D cell-derived extracellular matrices

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KEYWORDS: Cell-derived matrix, Tumor model

INTRODUCTION: The use of cell-derived matrices (CDM) is a promising alternative to decellularized tissues/organs, consisting of a complex assembly of macromolecules. 3D cell-cultured PLA microparticles combined with macromolecular crowding (MMC) effect, offers the possibility to tailor-made bioactive materials for disease modeling applications. We propose CDMs as potential colorectal tumor models for personalized medicine by mimicking tissue microenvironment properties[1].

METHODS: CDMs are produced by seeding human mesenchymal stem cells on fibronectin-coated PLA microparticles2 and cultured in presence of macromolecular crowders (MMCs). Obtained CDMs are biochemically characterized by immunostaining and mass spectrometry; gene expression by qRT-PCR and RNA-sequencing; and mechanical properties by atomic force microscopy. Decellularized and particle-free CDMs are recellularized with colorectal cancer cells and cancer associated fibroblasts (CAFs)3 to further characterize cell-cell interactions, gene expression and their CDM-remodeling potential. Obtained CDMs are compared with human colorectal tumor samples from patients.

RESULTS: MMC effect enhances protein deposition in CDMs. Fibrillary proteins collagen types I, III and fibronectin are CDMs' main constituents, resembling colon tumor extracellular matrix. Decellularized and particle-free CDMs were recellularized with colon cancer cells and CAFs. Cancer CDMs' characterization is taking place to develop an *in vitro* tumor model to understand cancer promoting mechanisms, develop patient- specific drug screening platforms and to identify potential therapeutic targets.

CONCLUSIONS: CDMs composition and the tunable matrix stiffness provides reproducible tissue microenvironment. By repopulating CDMs with cancer and stroma cells, we pretend to mimic native tissue structure and properties to obtain a promising platform for *in vitro* tumor model generation.

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Flagellated flexible magnetic microrobots

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Biohybrid microrobots are autonomous microdevices in which biological components are incorporated as propulsion source, structural components or loading units. Microbiorobotics is a fascinating field of research with promising applications in future and precision medicine due to non-invasive action, high biocompatibility and adaptability to complex *in vivo* conditions.

In this work, we first present biotemplated magnetic microrobots, referred to as IRONSperms, which were developed by electrostatic-based self-assembly of non-motile sperm cells and magnetic nanoparticles. This results in soft magnetic swimmers actuated by external magnetic fields emulating the motion of motile sperm cells. [1] Such microrobots are under 100 µm in length and have unique potential applications for therapy and diagnosis in healthcare. It is also demonstrated that the nanoparticle coating increases the acoustic impedance of the sperm cells and enables localization of clusters of IRONSperm using ultrasound feedback. [2] Finally, cytotoxicity tests show the biocompatibility of IRONSperms and the drug delivery capability is demonstrated by loading their organic body with a model anti-cancer drug. This work presents new insights into the development of a biocompatible, controllable, and detectable biohybrid magnetic microrobot for *in vivo* targeted therapy.

Mimicking the morphology and motion of spermatozoa, we also propose flagellated artificial magnetic microrobots based on 3D printing of elastomers. This allows the determined fabrication and controlled addition of magnetic segments. The 3D printed flagellated microrobots have the potential to swim in complex environments overcoming tight spaces and high viscosities, for instance. Furthermore, the bioprinting technique allows to engineer hybrid systems using cell-laden hydrogels as structural material.

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Engineering human Pluripotent Stem Cells (hPSCs) lines with CRISPR/Cas9 for constitutive and inducible Knock Out in Kidney Organoids.

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The possibility to generate organ-like cultures from human pluripotent stem cells (hPSCs), so called organoids, opens the door to immediate studies in human development and disease. Current limitations for the study of human disease using these 3D culture systems arise from technical challenges targeting specific loci during the process of organoid formation. In this regard, our laboratory has recently generated hPSCs lines sustaining for the inducible expression of Cas9 (iCas9) under the control of the endogenous TET/ON system by targeting the AVVS1 locus of undifferentiated hPSCs via TALEN mediated targeting. Profiting from this approach we have generated different hPSCs lines allowing for knock-out (KO) or knock-in (KI) applications in hPSCs derived kidney organoids.

To explore on the impact of KO genotypes in kidney differentiation we have generated constitutive knock-out hPSCs lines (stable KOs) for *loci* previously identified in congenital kidney disease (PAX2 and LHX1) as well as in clear cell renal cell carcinoma (VHL). This was achieved by the transfection of gRNAs into iCas9 hPSCs. To ascertain for the effect of this same genotypes in an inducible fashion we have generated hPSCs lines that constitutively express those same gRNAs in hPSC iCas9 lines. This approach allows for the generation of inducible knock-out (iKO) upon doxycycline treatment. In this manner, we have established a dual approach for the interrogation of knock-out associated phenotypes in a constitutive fashion (stable KO lines) or during kidney organoid differentiation (iKO lines). Our results show how accumulation of KO mutations at target genes is traduced into downregulation of their mRNA and protein expression levels. Characterization of phenotypic differences using confocal microscopy analysis, Western Blot and quantitative real time PCR comparing stable and inducible KO genetic backgrounds show the usefulness of our engineered lines to understand the role of specific genes during kidney development and disease.

Peripheral cannabinoid type 1 receptor blockade enhances memory persistence in mice through an adrenergic mechanism

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Memory performance is sensitive to central and peripheral responses. Our group demonstrated that the peripheral cannabinoid type-1 receptor (CB1R) was involved in the memory consolidation impairment induced by stress. Here, we have evaluated the peripheral CB1R modulation on non-emotional memory persistence using the novel object-recognition test (NORT). We found that the peripherally restricted CB1R antagonist AM6545 exhibits a mnemonic effect in the NORT that was suppressed in adrenalectomized mice or when mice were pre-treated with the peripherally restricted β-adrenergic antagonist sotalol. Genetic CB1R deletion in dopamine β-hydroxylase-expressing cells also facilitated memory persistence further supporting a role of the adrenergic tone modulated by the endocannabinoid system in memory persistence modulation. Moreover, chemogenetic inhibition of neuronal vagus nerve activity reduced AM6545 memory enhancement, suggesting that peripheral AM6545 effect could be projected to the central nervous system through vagus nerve activation. In the brain, acute AM6545 treatment increased locus coeruleus activity as well as extracellular norepinephrine levels in the hippocampus. Such enhancement in the

noradrenergic axis was relevant to the mnemonic effect of AM6545 since intra-hippocampal injection of the β-adrenergic antagonist propranolol prevented the memory improvement. Furthermore, repeated AM6545 administration produced neuronal plastic and functional changes in the hippocampus.

Overall, these results reveal that the peripheral CB1R contributes to the modulation of memory persistence and hippocampal synaptic plasticity involving peripheral and central adrenergic/noradrenergic mechanisms.

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Promoting in situ cardiac regeneration through lactate-based biomaterials

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Lactate, an important metabolite during cardiogenesis and cardiac development, has been recently shown to promote cardiomyocyte proliferation and reprogramming towards a dedifferentiated stem cell-like state [1]. Non-myocytic cardiac fibroblasts (CFBs) have a key role during cardiac remodeling after myocardial infarction, where they become activated and release extracellular matrix proteins. However, excessive extracellular matrix deposition or myofibroblast activation is detrimental to cardiac repair.

The aim of this work is to elucidate the effect of lactate on CFBs for a complete interpretation of its potential proregenerative capabilities. Then, we developed bioactive lactate-releasing scaffolds for cardiac regeneration.

METHODS: CFBs metabolic activity and proliferation were assessed. The inflammatory and fibrotic responses to exogenous lactate were investigated in terms of cytokine and collagen production, migration assays and myofibroblast differentiation. Then, CFBs were cultured on polylactic-acid (PLA) scaffolds fabricated by electrospinning and physiochemically characterized (SEM, DSC, lactate release, tensile testing).

RESULTS: Our results indicate that lactate does not affect fibroblast proliferation, migration, collagen production, or activation and significantly reduces the expression of detrimental cytokines for cardiac repair. Furthermore, our PLA scaffolds are amorphous, biodegradable, nanofibrous materials that release lactate in a sustained manner. They do not affect CFB viability and promote cell attachment.

DISCUSSION: Altogether, this study further supports the prospective use of lactate as a bioactive signal in new endogenous cardiac regeneration therapies. Thus, PLA patches that release lactate as a major product from its breakdown can then be used as an effective source of lactate for alleviating the aftermath of myocardial infarction.

Keywords: Lactate, cardiac fibroblasts, in situ cardiac regeneration

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Characterization of the intrinsic changes induced by the modulation of neuronal activity after central nervous system injury

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The central nervous system (CNS) presents an intrinsic inability to regenerate upon injury. As a result, patients with this kind of injuries suffer from permanent disabilities. In spite of many efforts, an effective treatment for this condition is still lacking. Activity-based therapies represent the only approach presenting slight success in patients. However, the molecular mechanisms responsible for this recovery are still unknown. We believe that defining these mechanisms will help to identify new targets for CNS injuries treatment.

We hypothesize that neuronal activity is able to induce intrinsic changes in injured neurons, promoting regeneration. Thus, we manipulate neuronal activity in specific neurons through optogenetic and chemogenetic tools in injured mice and characterize them at the molecular level. We work in parallel using two different systems: a pro-regenerative, the dorsal root ganglia neurons (DRGN), and a refractory to regrowth, the corticospinal motorneurons (CSMN). Regeneration and recovery are assessed by histological and sensorimotor function evaluation. In terms of molecular studies, combined RNA-seq and ATAC-seq are performed using isolated neurons of interest: neurons that regenerate as a consequence of our treatment. For that, we have designed a method which combines fluorescent retrotracing of regenerating neurons with posterior FACS (fluorescence-activated cell sorting) isolation which allows us to successfully obtain them.

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Biomanufacturing platform development for the large-scale production of non- parenchymal cells towards the bioengineering of a porcine whole liver

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Liver transplantation represents the only option for patients with end stage liver disease (ESLD). Regrettably, this solution is limited by growing demand and insufficient supply of organs available for transplantation. Organ bioengineering is based upon the use of scaffolds with a preexisting architectural structure to mimic the complex microenvironment of the organs, which allows the seeding and survival of functional cells. Decellularization encompasses the techniques used to remove the cellular content of organs, preserving a scaffold composed of an intact extracellular matrix and vascular tree. Once decellularized, these scaffolds could be recellularized to create functional bioengineered organs able to be transplanted into recipient animals and, in the long-term, into patients with ESLD.

The main goal of this work is to generate the necessary amounts of cells required to re-establish a functional vasculature in bioengineered livers of clinically relevant size. To fulfill this goal, we are developing a robust, reproducible, and cost-effective platform for the large-scale expansion of non-parenchymal cells, namely bone marrow-derived mesenchymal stromal cells (MSCs), aortic smooth muscle cells (SMCs), and umbilical vein- derived endothelial cells (ECs) under dynamic culture conditions. Cells were isolated from 5kgs piglets, expanded under static conditions and characterized. Afterwards, the three non-parenchymal cells were cultured on microcarriers in spinner flasks to investigate the effect of different parameters such as microcarrier type, culture medium and the feeding regimen.

Overall, using the best condition studied for each cell type, cells reached maximum numbers of 15X106 of MSCs cultured using Fetal Bovine Serum (FBS)-supplemented medium, 20X106 of SMCs cultured with human Platelet Lysate (hPL)-supplemented medium, and 7.6X106 of ECs expanded with porcine endothelial growth medium, which corresponds to fold increase values in total cell number of 6, 11 and 3, respectively. Importantly, cells expanded under stirred conditions retained their phenotype in what concerns to the expression of a set of specific markers, as assessed by flow cytometry and immunofluorescence analysis. Finally, we were able to successfully expand MSCs and SMCs under fully controlled conditions in stirred tank bioreactors. Cells reached maximum numbers of 170X106 of MSCs, and 200X106 of SMCs using hPL-supplemented medium, which corresponds to fold increase values in total cell number of 17 and 33, respectively. Similarly to spinner flask cultures, cells expanded in stirred tank bioreactors retained their phenotypical identity, as confirmed by flow cytometry and immunofluorescence analysis. These results demonstrated the feasibility of expanding MSC, SMC and EC in a scalable microcarrier-based stirred culture system and could represent an important step toward the production of the required amounts of cells for the reconstruction of whole organs for transplantation purposes.

Key-words: Organ bioengineering; Decellularization; Spinner flasks; Stirred tank bioreactors; Recellularization

3D Bioprinting lung resident mesenchymal stromal cells in extracellular matrix hydrogels

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Our aim is to investigate how bioprinting and 3D culturing lung resident MSCs in lung-derived extracellular matrix (ECM) hydrogels produce changes in cell behavior.

METHODS: Rat primary lung resident MSCs were bioprinted and 3D cultured in porcine lung ECM hydrogels presenting a stiffness of 0.7kPa. After seven days of 3D culture, cells were harvested from the scaffolds. Cell adhesion and actin/paxillin staining tests were conducted with the harvested and control cells by seeding them onto specific well-plates and allowed to attach them to the plate for 2h. The expression of surface chemokine receptor CXCR4 was quantified by qRT-PCR.

RESULTS: Cells harvested from the lung ECM hydrogel scaffolds formed focal adhesions 2-fold longer. Moreover, 10-fold more cells were adhered to the substrate after 2h. Finally, the expression of CXCR4 chemokine receptor showed a more than 20-fold increase in the preconditioned cells.

DISCUSSION: The data indicate that culturing lung MSCs in the ECM has major impact in their adhesion capacity and in the expression of one the main receptors involved in several relevant processes *in vivo*. Thus, lung ECM-derived hydrogels have the potential to be used as a scaffold to develop novel *in vitro* models to better understand mechanisms in MSCs.

Study of the interplay between glucose metabolism and SARS- CoV-2 infection in kidney organoids

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Severe acute respiratory syndrome 2 (SARS-CoV-2) infection leads to a high risk of hospitalization and mortality in diabetic patients. SARS-CoV-2 binds to angiotensin-converting enzyme 2 (ACE2) receptor, which is expressed in key metabolic organs such as pancreas, muscle, heart, adipose tissue, the small intestine, and the kidneys. As a result, it is likely that SARS-CoV- 2 may cause alterations of glucose metabolism that could complicate the pathophysiology of preexisting diabetes or lead to new mechanisms of disease.

We have previously showed that SARS-CoV-2 can directly infect engineered human blood vessel organoids and human kidney organoids. Here, we infected human kidney organoids cultured under hyperglycaemic condition. We found that kidney organoids under hyperglycaemic condition have higher expression of ACE2 by western blot, Immunofluorescence and RT-PCR. Furthermore, viral loads were determined by qRT-PCR showing higher expression in kidney organoids under hyperglycaemic condition. Thus, to identify new mechanisms which could explain the poor prognosis of diabetic patients infected by SARS-CoV-2 and the alteration in glucose metabolism, we performed scRNA-sequencing and bioinformatics analyses in kidney organoids under hyperglycaemic conditions versus control.

Together, our results provide evidence that SARS-CoV-2 infection altered glucose metabolism and support the use of kidney organoids as a platform to investigate the cellular susceptibility, disease mechanisms, and treatment strategies for SARS-CoV-2 infection in hyperglycaemic condition.

Combisomes Solve the Dilemma of Polymer-based Cell-Mimetic Membranes

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Natural membranes achieve an incredibly rich functionality by the self-assembly of different building blocks, mainly phospholipids and proteins at an almost invariable thickness (5±1 nm). Remarkably, despite their minute thickness and flexibility, natural membranes are incredibly stable. The combination of these seemingly antagonistic properties, i.e. flexibility and stability, is the key for life to exist. The thickness and flexibility have been mimicked by assembly of lipids into liposomes. Nonetheless, liposomes lack stability to environmental conditions, severely limiting their use for advanced functions. Polymersomes from amphiphilic block copolymers display an enhanced mechanical stability due to the entanglement of the hydrophobic blocks, but at the expense of thickness well above the natural ones and an almost complete stall of the dynamics (viscosity, diffusion, flip-flop) compared to biological membranes. Furthermore, the mismatch between the membrane thickness and the size of transmembrane proteins has been the main obstacle hampering the integration of natural bioreceptors in polymersomes. In this poster, I will present our advances in cell mimetic membranes based on amphiphilic comb polymers. The polymers consist of a hydrophilic highly flexible backbone to which fatty-acid-like side groups are appended. In water, these amphiphilic comb polymers self-assemble into biomimetic vesicles –combisomes. We developed an accelerated iterative combinatorial synthesis to generate a library with systematic structural variation. This allowed us to elucidate how to program the thickness, stability, and flexibility in the molecular structure and topology of the polymer and in this way solve the dilemma of combining stability with extreme flexibility and biomimetic thickness. Contrary to block copolymer, no entanglement of hydrophobic domains occurs, thus the thickness and flexibility of our membrane mimic closely matches those of their natural counterparts. This is demonstrated by structural analysis of the bulk assembly and lyotropic phases as well as by the insertion of transmembrane proteins. Our combisomes provide the technical platform to bring complexity and dynamics of nature to synthetic cells and design interactive protocells. Moreover, they promise diverse applications in medicine. These vesicles can be designed to provide a versatile platform for cargo carriers or antimicrobial agents.

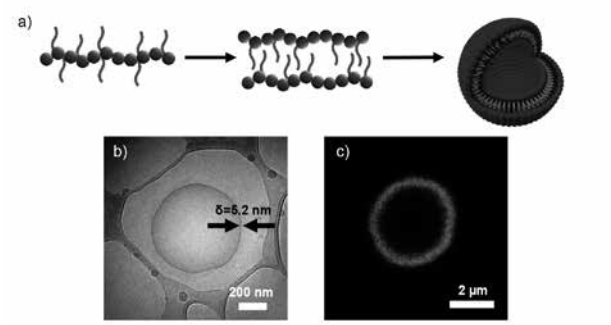

Figure 1. a) Self-assembly of comb polymer into flexible stable combisome, observed by b) cryogenic transmission electron microscopy and c) confocal laser scanning microscopy

Personalized Bioactive and Biodegradable Implants for Maxillofacial Bone Regeneration

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Metallic meshes or degradable membranes filled with different bone grafts are commonly used to treat maxillary bone defects. Metallic meshes, due to their mechanical properties, can preserve the stability of the implanted graft along the regeneration process, while degradable membranes present lower capacity to maintain the stability. Moreover, in the case of metallic meshes is required a second surgery to remove the mesh after the regeneration process, increasing the possibility of side effects. For this reason, the objective of this industrial project is to develop patient specific, bioactive and biodegradable implants to treat maxillofacial bone defects.

METHODS: Different bioactive Calcium Phosphate-based microparticles (MPs), were synthetized and dispersed into Polycaprolactone (PCL). Mixtures obtained were used as a raw material to fabricate 3D printed scaffolds by fused deposition, allowing the production of complex geometries and porosities. Scaffolds degradation profile at *in vitro* physiological conditions was studied. Biological characterization was tested with human mesenchymal stem cells (hMSCs) and gingival fibroblasts (hGFib). Moreover, key proteins produced by hMSCs such us alkaline phosphatase and vascular endothelial growth factor were quantified.

RESULTS: Parallel patterned scaffolds with interconnected macro porosity were obtained. Scaffolds showed a homogeneous dispersion of MPs phase into PCL by SEM cross section images. No cytotoxicity on hMSC was detected following ISO10993 recommendations. hMSCs and hGFib seeded on scaffolds showed a good biocompatible behavior according to metabolic activity results and fluorescent confocal images. Different clinical cases of maxillary defects with complex geometries were studied, obtaining a correct fit of the printed implants on their corresponding defect models, verifying the personalization of the implants produced and their volume preservation.

DISCUSSION & CONCLUSIONS: Our results suggest that these biodegradable platforms could be a promising approach as a substitute of current therapies for treating maxillofacial bone defects. Their high biocompatibility with hGFib, may prevent drawbacks such as mesh exposure. Moreover, they are obtained by 3D printing thus allowing a high level of personalization to perfectly fit different defect geometries and maintaining stability during the regeneration process.

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Development of ion-releasing platforms for wound healing applications

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Chronic wounds represent a major burden in human society, with high costs associated with extensive care treatments. Research has focussed on the development of new wound healing devices. However, a device that enables fast-effective closure, low cost, and scalability is still missing.

Ions such as calcium and zinc are essential for skin homeostasis. Calcium regulates a plethora of skin vital functions. We have shown that calcium releasing platforms such as calcium phosphate nanoparticles (NPs) stimulate *in vitro* and *in vivo* wound healing [1,2]. On the other hand, zinc deficiencies are associated with impaired wound healing. zinc 's antimicrobial properties make this ion a potential substitute for antibiotics.

This work aims to develop different ion releasing platforms based on nanocomposites for local and sustained ion release at the wound site.

METHODS: NPs incorporating Zn2+ and Ca2+ were synthesized by double emulsification, nanoprecipitation and sol-gel synthesis. Ion release was measured by colorimetric methods. NPs were encapsulated in 3D printed gelatine scaffolds.

RESULTS: NPs were synthesized with diameters ~300 nm and narrow size dispersity. Both ions were successfully incorporated into the carrier NPs. Scaffolds showed good particle distribution. The release of zinc ions showed enhanced antibacterial activity.

DISCUSSION AND CONCLUSION: Ion releasing platforms were successfully produced. Their composition, size, and ion release profiles indicate their potential use in soft tissue applications such as wound healing therapies.

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