Bioengineering for Regenerative Therapies

14th IBEC Symposium

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Institute for Bioengineering of Catalonia

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Welcome to IBEC's 14th annual symposium

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

Although the symposium will be held online, due to COVID-19, we have incorporated some new features in order to improve your user's experience and make it more participative.

We have included small poster sessions rooms to stimulate discussions and a virtual space where participants are able to to personalise and upload all kinds of material (both visual and written) to show the attendees their work. Two prizes will be given to this new type of format, one by a scientific committee and one by the public.

Thank you very much for participating in the Symposium!

Josep Samitier
Director

Bioengineering for Regenerative Therapies

14th IBEC Symposium

Programme

Wednesday 27th October · 14th IBEC Symposium for Regenerative Therapies

09:15 - 10:00	Opening Ceremony
10:00 - 10:30	Keynote speaker IBEC's Evolution and Scientific Highlights. **Josep Samitier** · *Institute for Bioengineering of Catalonia (IBEC)*
10:30 - 10:45	Keynote speaker Presentation of the IBEC ISCIII Unit for organoid production, biofabrication and organ-on-a-chip: bioengineering services for health and personalized medicine. **Núria Montserrat** · *Institute for Bioengineering of Catalonia (IBEC)*
10:45 - 11:20	Keynote speaker Identifying mechanisms relevant for cardiac remodeling and repair. **Eva van Rooij** · Hubrecth Institute
11:20 - 11:40	Keynote speaker 3D live imaging of mouse and human embryos using endogenous signals. **Samuel Ojosnegros** · *Institute for Bioengineering of Catalonia (IBEC)*
11:40	Flash Presentations
16:00	Online Poster Session

Thursday 28th October · 14th IBEC Symposium for for Regenerative Therapies

10:00	Online Poster Session
15:00 - 15:25	Keynote speaker Understanding the role of the extracellular matrix: from elasticity to viscoelasticity. **Alberto Elosegui-Artola** · *The Francis Crick Institute*
15:25 - 16:00	Keynote speaker Regenerative Medicine and Tissue Engineering: Lost in Clinical Translation? **Frank P.Luyten** · *KU Leuven, Belgium & Medical/Scientific Director of RegmedXB*
16:00 - 17:00	Flash Presentations
17:00 - 17:35	Keynote speaker Biomanufacturing of vascularized human tissues. **Jennifer A. Lewis** · *Harvard University*
17:35 - 17:40	Sustainability Committee presentation
17:40 - 17:50	PhD Committee presentation
17:50 - 18:00	Postdoc Committee presentation
18:00	Awards and closing ceremony

Keynote Lectures

Wednesday 27th October · 10:00

IBEC's Evolution and Scientific Highlights

Josep Samitier

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

The year in which science was more demanded than ever, IBEC was at the forefront. With the outbreak of COVID19 pandemic we showed, once again, that excellent research in bioengineering is at the service of society, helping to improve health and to generate wealth.

This presentation is a round-up of the scientific, innovation, and institutional highlights, but also a prove of the extraordinary evolution of IBEC in the last years.

In 2020, IBEC researchers achieved their own record of 184 indexed scientific papers. And in the first 3 quarters of 2021 we have already reached 182 publications. Moreover, IBEC scientists such as Nuria Montserrat, Paul Verschure, Javier Ramón, Pere Roca-Cusachs, Raimon Jané, Santiago Marco or Giuseppe Battaglia reoriented their research, putting bioengineering against SARS-CoV-2 at the centre of their efforts, and leading to the discovery of a drug blocking coronavirus, developing methods to evaluate the impact of lockdowns on mental health or exploring new technologies to fight sequela after coronavirus infection. COVID-19 was the topic selected for IBEC's Faster Future fundraising campaign. In June 2021 bioengineering against COVID-19 receives a new boost thanks to "La Marató", which funded 3 projects by Prof. Nuria Montserrat, Santi Marco and Beatriz Giraldo to deepen our understanding of the disease and its possible therapeutic solutions, study improvements in patient care processes, develop a system to predict the evolution of the respiratory system, and advance in the treatment of patients with pneumonia derived from COVID19.

At the same time, IBEC managed to keep on going its research against diseases besides COVID19. With substantial scientific contributions against cancer, Alzheimer or bacterial infections, IBEC researchers were able to further contribute to our main mission: to search for bioengineering solutions which can help improving global health challenges.

Since last symposium IBEC researchers were, once again, awarded for their talent and excellence in research. César Rodríguez-Emmenegger from DWI-Leibniz Institute for Interactive Materials was selected as new ICREA Research Professor, accounting for a total number of 9 ICREA Research Professors and 2 ICREA Academia Awardees at IBEC. Nuria Montserrat, ICREA Research Professor and Group Leader at IBEC received the prestigious ERC Consolidator Grant, and became new member of EMBO, joining the elite in European biology. Nuria Montserrat was also awarded the "Constantes y Vitales" and National

Research Award for Young Talent Prizes. Marino Arroyo, associated researcher at IBEC, was honoured with an ICREA Academia award and IBEC Group Leader Silvia Muro was elected to join the American Institute for Medical and Biological Engineering (AIMBE). Roger Oria, former PhD student of Pere Roca-Cusachs lab won the XXIV Doctors' Senate Award of the University of Barcelona (UB) for his thesis on mechanobiology.

Regarding the attraction and retention of talent, we have incorporated one new junior group leader, Dr. Irene Marco in the framework of the BIST research program on chemical biology supported by la Caixa Foundation, and have launched the first call for the talent retention programme to incorporate senior researchers to our groups.

IBEC also performed at the highest level in clinical collaborations and technology transfer. This year, we also contributed, together with hospitals, to significant advances not only against COVID19, but also against childhood cancer or to a protocol for bioengineered implants for infarcted hearts. As a recognition of our relevance in the field, the Carlos III Health Institute granted Nuria Montserrat, the coordination of the National Platform of Biobanks and Biomodels, being one of the nodes of the platform located as well at IBEC.

We have also reinforced our strategic collaboration with The Vall d'Hebron Research Institute, with the organization of two translational conferences. Regarding technology transfer, in 2020, IBEC also submitted 6 new patents, and we have also signed two license contracts with companies, while in 2021 already 2 patents have been submitted.

The last year has also been a great year concerning our institutional alliances. We have continued our strategic collaboration with the Institute for Complex Molecular Systems (ICMS), from the Eindhoven University of Technology (TU/e), with our joint symposium organized on 26th October 2021. We also kept working with MIT in the field of engineering living systems. In this sense, together with EMBL, we organized the Barcelona Satellite event of the M-CELS workshop organized by the Center for Emergent Behaviors of Integrated Cellular Systems in June 2021.

Regarding social impact, we are proud to have reached millions of people with our press and communication activities, hundreds of students with our education and outreach activities and to have been able to launch an initiative with young creative talent, which will bring to life IBBI, the first figure of a Bioengineering Superhero. We have created a new are of Open Science, that will foster IBEC institutional activities regarding open access, open data, science education and citizen engagement.

Finally, I would like to specially thank all the IBEC community for their essential work, resilience, and commitment in this difficult time for everybody. It has been a year in which we can proudly say that scientists respond fast and without hesitation when world-wide health emergency occurs.

Josep Samitier

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

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) since 2015 and member of the Institut d'Estudis Catalans (IEC, Catalan Academia) since 2016. He is founder and coordinator of the Spanish Platform on Nanomedicine (Nanomed Spain) and member of the European Platform on Nanomedicine from 2005. He was founder and member of the EIT Health Supervisory Board from 2015 until 2019. Prof. Samitier has also been scientific advisor for a programme of the government of Argentina to foster nanotechnologies among SMEs and part of the Multidisciplinary Assessment Committee Canada Foundation for Innovation 2017 Innovation Fund competition. He 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 (OECD).

Prof. Samitier also has a long track record of participation in management activities, always holding positions of responsibility: leader of the Spanish node and member of the executive committee in the winning proposal Innolife for the Knowledge and Innovation Community (KIC) on healthy living and active ageing selected by the European Institute of Technology (EIT) in December 2014, director of the "Health Campus of Excellence" HUBc (University of Barcelona) from 2010 until 2013, associate director of the Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN) since its creation in 2007 until 2011, deputy head (CIBER-BBN) from 2006 until 2010, vice-rector of Research and Innovation, acting rector of the University of Barcelona (UB) from 2005 to 2008 and deputy head of the Barcelona Science Park (PCB) from 2002 to 2005.

Apart from that, Prof. Samitier is member of the international committee of the International Society for BioMEMS and Nanotechnology, TNT Conferences, Int. Steering member of Engin. International Engineering Living Systems workshops (2016 Chicago-2018 Chicago – 2019 Beijing), member on the editorial board of the Journal of Nanoscience and Nanotechnology*, editor in chief of Biomimetics (MDPI), Member of the scientific advisory board of GADEA Ciencia, member of the Strategic Committee ALINNSA from Spanish Ministry of Science, Spanish, academic member of the EIT Health Supervisory Board and member of the international scientific committee of ITAV (Toulouse), Cancéropole CLARA (Lyon), INESC Microsystems and Nanotechnologies (Lisbon) Lermit (France), Fraunhofer IBMT (Berlin-St Ingblert), CTB-UPM (Madrid), UC3M-Univ. Carlos III (Madrid).*

He received the Barcelona City Prize of Technology in 2003. In 2020, he was awarded by the Catalan Government with the Narcís Monturiol medal for his contribution to science and technology.

Presentation of the IBEC ISCIII Unit for organoid production, biofabrication and organ-on-a-chip: bioengineering services for health and personalized medicine

Núria Montserrat

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

The Spanish National Platform for Biobanks and Biomodels (ISCIII-BBP) coordinated by the Institute for Bioengineering of Catalonia (IBEC) has the vision to become a national and international model for international clinical and research institutions, administrations, and companies. Through its unique composition the ISCIII PBB is an infrastructure able to perform, with a scientific, technological and regulatory perspective, a complete preclinical validation from the design to the product to its *in vivo* validation. To achieve this goal the coordination from IBEC has structured the 41 UNITS of the Platform in four different HUBS to ensure a transversal role throughout the entire R&D&I value chain including: 3D BIOMODELS, BIOBANKS, 3D PRINTING, ANIMAL MODELS. Importantly, IBEC counts with all the infrastructure and expertise to provide the highest level of quality and state-of-the art services to facilitate and advance the definition of the best therapy for the patient and thus close the loop from basic research to the realization of clinical translation. In this talk we will explain the vision and mission of the ISCIII-BBP as well as to provide a comprohensive overview on current threats and challenges on how bioengineering strategies will help to bridge the gap between the discoveries from the IBEC UNIT of the ISCIII-BBP to effectively bring “patients in the lab".

Núria Montserrat

Institute for Bioengineering of Catalonia (IBEC)

Nuria Montserrat *became interested in organ regeneration and stem cells during her master and PhD training that finished in 2006. The same year she got a Postdoctoral fellowship from the Fundaçao para a Ciência e Tecnología (Portugal). In 2007 she moved as a post-doctoral researcher at the Hospital of Santa Creu i Sant Pau in Barcelona.*

In 2008 she joined the Center of Regenerative Medicine of Barcelona (CMRB) thanks to the support of a Juan de la Cierva fellowship under the direction of Dr. Izpisúa Belmonte. In 2010 she first co-authored how to reprogram cord blood stem cells for the first time (Nature Protocols, 2010). Then she first-coauthor the first work deriving iPSCs with new factors (Cell Stem Cell, 2013). She also collaborated in projects aimed to characterize the genomic integrity of human iPSCs as well as their differentiation towards different lineages for applications in human disease modeling (Stem Cells, 2011; Nature, 2012; Nature, 2012, Nature Communications, 2014). She has first co-authored how the reactivation of endogenous pathways can be artificially reactivated and promote heart regeneration in mammals (Cell Stem Cell, 2014).

Her expertise in the fields of somatic reprogramming and organ regeneration was recognized with an awarded an ERC Starting Grant by 2014 which allowed her to become Junior group leader at the Institute of Bioengineering of Catalonia (IBEC).

In January 2015 she got a Ramon y Cajal fellowship (first ranked candidate, 100/100 points) and since 2019 she is an ICREA Research Professor and Senior Group Leader at IBEC. During these years her findings in the field of Regenerative Medicine led to the derivation, for the first time, of cardiac grafts from human pluripotent stem cells and decellularized cardiac myocardium (Biomaterials, 2016), and the derivation of renal analogues with 3D bioprinting (Materials Today, 2017) and vascularized kidney organoids from hPSCs (Nature Materials, 2019).

She has recently co-led on the application of kidney organoid technology to model SARS-CoV-2 infections (Cell, 2020) identifying a therapeutic compound that nowadays is under clinical trial in COVID19 patients (The Lancet Respiratory Medicine, 2020; EMBO Molecular Medicine, 2020). Their Cell paper has been highlighted as a Research Highlight in both Nature Reviews Nephrology and in the Nature journal, also attracting remarkable attention (more than 200 national press releases, and 30 radio/television) being awarded as the Best biomedical research publication 2020 (Constantes y Vitales Prize).

With the aim to further understand on the central role of metabolism in renal fate and damage protection she has recently co-led the first work on the identification of metabolic regulators protecting the renal tubule from acute injury exploiting kidney organoid technology (Cell Metabolism, 2020). In sum, from 2015, her work has attracted almost 8 M of direct competitive funding and around 1.2 M in personnel related grants, from both Spanish and European institutions.

All these efforts have been recently awarded with the prestigious EMBO Young Investigator Prize. In December 2020 the ERC has recognized all these efforts and she have been awarded with the prestigious ERC Consolidator Grant to study the interplay between mechanobiology and metabolism during human kidney development and disease.

Her commitment to scientific dissemination and communication has allowed her to participate in more than 300 outreach activities to promote our research activities to the general public, with a particular focus to young girls and woman in science. For this reason, she was selected as Commissioner of the first City and Science Biennial of Barcelona in 2019 being re-elected for the second edition (June, 2021).

Identifying mechanisms relevant for cardiac remodeling and repair

Eva van Rooij

Hubrecht Institute, KNAW;
Dept. Of Cardiology, UMC Utrecht

Chronic and acute stress to the heart results in a pathological remodeling response accompanied by hypertrophy, fibrosis, myocyte apoptosis and eventual death from pump failure and arrhythmias. While genome-wide transcriptome analysis on diseased tissues has greatly advanced our understanding of the regulatory networks that drive pathological changes in the heart, this approach has been disadvantaged by the fact that the signals are derived from tissue homogenates inherently diluting local or cellular signals. Recent developments in RNA amplification strategies provide the opportunity to use small amounts of input RNA for genome-wide sequencing, allowing for in-depth sequencing of small pieces of tissues or even cells. Combining this with the use of iPSCs and gene editing strategies, provide an ideal platform for identifying and studying new disease driving mechanisms relevant for cardiac biology and disease.

Eva van Rooij

Hubrecth Institute

Eva van Rooij *attended University Hospital Maastricht in the Netherlands where she received a Ph.D. at the department of Cardiology. She then went on to complete postdoctoral training in Molecular Biology at UT Southwestern Medical Center in the lab of Dr. Eric Olson where she served as lead scientist in the studies that linked microRNAs to cardiovascular disease. In her current work she combines high-end sequencing technologies, stem cells, mouse genetics, animal models of heart disease, and molecular biology to identify the important pathways for cardiac remodeling and repair that could aid in the development of new therapies for heart disease..*

3D live imaging of mouse and human embryos using endogenous signals

Samuel Ojosnegros

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

Advanced optical imaging imposes restrictions to the sample regarding size, mounting or labelling However, fulfilling the stringent criteria required by many imaging methods, especially by fluorescent optical microscopy, often compromises the physiological configuration of the sample. Such is the case of mammalian embryos, including humans, during early development. It is not possible to generate transgenic human embryos fluorescently labelled, which makes 3D live imaging challenging. Furthermore, mammalian embryos implant into the uterus of the adult mother thus becoming inaccessible to live imaging. These reasons have severely limited the understanding of the development of our own species. In our laboratory, we combine bioengineering and optical methods to provide solutions to these limitations. We use multiphoton illumination to provide a specific and safe source of light to excite fluorescent metabolites, which are naturally present in the mammalian embryo. We unmix the raw and overlapping output spectra using a novel phasor approach. This allows us to reconstruct 3D images and movies of live embryos in label-free conditions, based on their metabolic signature. We have also designed a matrix which allows the embryo to implant ex vivo, thus acting as a surrogate of the mother uterus. The system is amenable to optical (label-free) microscopy.

Both technologies have revealed new dimensions of embryo development (e.g. spectral, mechanical). Accessing this novel information has allowed us to make discoveries such as the mechanosensitivity of the implanting embryo, develop new reagents to foster embryo implantation, or design devices for the diagnostic of embryos for the fertility industry. Our group is the first Open Innovation Laboratory, a new type of research lab at IBEC born with the transfer of technology in the core of its mission. Our goal is to make our technology accessible to society and to the health challenges we face in our time. To that purpose, we collaborate with the private sector, ranging from large pharma and engineering industry to small biotech or venture capital. We license technology or incorporate new companies to connect fundamental research with market and clinical needs.

Samuel Ojosnegros

Institute for Bioengineering of Catalonia (IBEC)

Samuel Ojosnegros *is the head of the Bioengineering for Reproductive Health laboratory, the first Open Innovation laboratory at IBEC. The research in his group focuses on the process of invasive implantation of mammalian – mouse and human – embryos and the rich cell-to-cell communication taking place during this event. The embryo invasion of the uterine tissue and the placenta formation is a crucial process for human development and reproduction, but still elusive to experimentation because of its inaccessibility. His group has combined advanced label-free imaging methods (spectroscopy and scattered light), with bioengineering methods to develop proprietary technology to efficiently culture pre-implantation embryos and allow them to implant ex vivo.*

His proprietary technology has led to several patents licensed by the pharma industry and to the incorporation of biotech start-up companies. His lab has received investment from private parties, including multinational companies, small biotechs or venture capital. Samuel did his PhD at the Centro Severo Ochoa, from CSIC in Madrid. He then performed two postdoctoral stages at the Biosystems Department at ETH-Zurich and at Caltech. He returned to Barcelona to lead the Open Innovation lab, a new initiative to strengthen the bonds between basic research and the industry.

Understanding the role of the extracellular matrix: from elasticity to viscoelasticity

Alberto Elosegui-Artola

Francis Crick Institute, London, UK
King's College London, London, UK

The mechanical properties of the extracellular matrix (ECM) regulate processes during development, cancer and wound healing. The vast majority of research efforts in this field have been focused on ECM's elasticity as a leading determinant of cell and tissue behaviour. However, the ECM is not merely elastic but is instead both viscous and elastic. Due to its viscoelastic nature, the ECM response to mechanical loads is inherently dynamic and evolves with time, independently of matrix degradation or remodelling. Despite the universality of ECM's viscoelasticity, how viscoelasticity affects tissue function is unknown. I will present our results where we show that the passive viscoelastic properties of the ECM regulate multicellular tissues spatial and temporal organization. By combining computational modelling with experiments, we confirm that the viscoelastic properties of the matrix regulate spherical tissues symmetry breaking, invasion and branching. Furthermore, ECM viscoelasticity controls epithelial to mesenchymal transition and tumour growth both *in vitro* and *in vivo*. Altogether, our results demonstrate the role of viscoelasticity in symmetry breaking instabilities associated with fingering, a fundamental process in morphogenesis and oncogenesis, and suggest ways of controlling tissue form using the extracellular matrix.

Alberto Elosegui-Artola

Francis Crick Institute

Alberto Elosegui-Artola *obtained a PhD in Biomedical Engineering from the University of Navarra (Spain) in 2012. After graduation, he moved to Barcelona to the laboratory of Pere Roca-Cusachs at IBEC to pursue his postdoctoral studies. During this time, he contributed to the identification of novel molecular mechanisms that explains how cells sense and respond to mechanical properties. In 2017, he was funded with a Marie-Skłodowska Curie fellowship to continue his research training in David J. Mooney's laboratory at Harvard University. Here, he examined the influence of the extracellular matrix viscoelasticity in cell and tissue response. In 2020, he was awarded an ERC Starting Grant and he has started his lab at the Francis Crick Institute in 2021 in a joint appointment with the Physics Department at King's College London. He integrates physics, engineering and biology to study the role of mechanics in living tissues.*

Thursday 28th October · 15:25

Regenerative Medicine and Tissue Engineering: Lost in Clinical Translation?

Frank P. Luyten, MD, PhD

Medical/Scientific Director RegmedXB, Maastricht, Netherlands & Prof. Em., Dept. of Development & Regeneration, KU Leuven, Belgium

The field of Regenerative Medicine and Tissue engineering aims to restore the function of damaged or diseased tissues or organs, ultimately replacing them when required. Despite significant advances in the last decade, in particular in the field of stem cell biology and biomaterials, major hurdles remain with respect to Clinical Translation. Indeed, the impact of new cost effective regmed treatment strategies for the patient and society has been quite modest so far.

In our quest to translate new cell-based treatment strategies to the clinic, and the market, we have encountered many challenges including a poor understanding of the mechanisms of action, scaling and manufacturing, regulatory requirements, defining the patient, clinical trial design and execution and business models. Pioneering examples of cell based Advanced Therapy Medical Products for skeletal indications that illustrate some of these aspects will be discussed in more detail. Future perspectives will be presented.

Frank P. Luyten, MD, PhD

Professor Emeritus at the KU Leuven
Medical/Scientific Director of RegmedXB

Frank P. Luyten *is Professor Emeritus at the KU Leuven, Belgium & Medical/Scientific Director of RegmedXB, Maastricht, Netherlands.*

After his Residency training in Internal Medicine (1980-1983) and Rheumatology (1983-1986), he spent considerable time in basic science, a postdoctoral training in cell and developmental biology of the skeleton with specific attention for the role of the Bone Morphogenetic Proteins (BMP) under the mentorship of AH Reddi at the National Institute of Dental Research (now NIDCR), National Institutes of Health (NIH) in Bethesda, USA, between 1986 and 1991.

*He became in 1992 Chief at the NIDR, NIH, Bethesda, MD, USA (Developmental Biology Unit, Bone Research Branch). During this period (1992-1997), he made significant contributions with seminal discoveries such as the Cartilage Derived Morphogenetic proteins 1,2,3 (CDMPs), now called GDF5/6/7, novel members of the BMP family (*J Biol Chem 1994*) and Frzb, the first Wnt antagonist (*J Biol Chem 1994; Cell 1997; PNAS 1997*). He was successful in investigating the role of CDMPs in the "human model", by the identification of the human skeletal phenotypes associated with mutations in CDMP1/GDF5 reported in several highly cited papers in Nature Genetics (*1996, 1997*).*

At the peak of this successful research phase, he decided to take on the next challenge, i.e. moving these findings closer to the patient, a translational research effort from bench to bedside, by accepting the position of Head of the Division of Rheumatology at the University Hospitals KU Leuven, Belgium. The grand plan was to establish a modern Rheumatology department with a highly integrated team of basic scientists, translational and clinical researchers. To achieve this, he established the Skeletal Biology and Engineering research Center by incorporating clinical rheumatology with research units in developmental and stem cell biology and genetics, tissue homeostasis and disease, skeletal biology and physiology as well as mechanobiology and tissue engineering. The SBE Research center (http://gbiomed.kuleuven.be/english/research/50000640/sberc) evolved into an internationally well recognized center of musculoskeletal research obtaining the EULAR recognition in 2017- 2021 as a Center of Excellence.

As a confirmation of his international Scientific Excellence, he obtained in 2012 a prestigious ERC advanced grant for REJOIND. This substantial financial support served to build the scientific basis for a next goal i.e. manufacturing of a biological joint.).

He demonstrated Leadership in Regenerative Medicine and Tissue Engineering. He co- founded and became director of an interdisciplinary platform for Skeletal Tissue Engineering, called Prometheus (http://www.kuleuven.be/prometheus), to foster close interactions between biologists, engineers and clinicians resulting in joint publications and novel tissue engineering and regenerative medicine approaches.

He is principal inventor of more than 15 granted patents/patent families.

Moreover, he can claim to belong to the few academicians that can bridge the gap between bench and bedside and make the transition to biotech and product development successful. As co-founder of TiGenix, together with his business partner Gil Beyen, they guided the company through several capital increases to a successful IPO in 2009 (TIG, Euronext Brussels and NASDAQ). More importantly, this effort led to the development and central registration at the European Medicines Agency (www.europe.ema.eu) of the first ATMP- cellular product, ChondroCelect™ for the repair of damaged joint surface defects. In doing this, he made critical contributions to the development of the regulatory path for Advanced Therapeutic Medicinal Products (ATMP) in Europe, as no regulatory frame existed at the start of this endeavor. Taken together, I believe he has demonstrated that I can move boundaries, and capable of mobilizing substantial resources, both human and financial, towards the goals set and finish "the job". The company obtained Central Market Approval in Europe for a second allogenic stem cell product AlofiselTM and was subsequently acquired in June 2018 by Takeda.

Prof. Luyten is/was member of the Board of Directors of several Biotech companies including PharmaCell, Maastricht, Netherlands, and Neuroplast, Geleen, NL. He is also Medical and Scientific Advisor of Biotech and Pharma Companies.

Prof. Luyten was appointed as a member of the European Commission's Scientific Panel for Health (SPH). The Scientific Panel for Health is a science-led stakeholder platform which provides a coherent scientific focused analysis of research and innovation bottlenecks and opportunities related to Life Sciences, contributes to the definition of its research and innovation priorities, and encourages Union-wide scientific participation in it. Through active cooperation with stakeholders, it aims to build capabilities and to foster knowledge sharing and stronger collaboration across the Union in this field.

Finally, he has recently been appointed as medical/scientific Director of REGMED-XB (https://www.regmedxb.com), an ambitious across border initiative started in the Netherlands and Flanders joining, focusing on the development and industrialization of the field of regenerative medicine.

Biomanufacturing of vascularized human tissues

Jennifer A. Lewis

Wyss Institute for Biologically Inspired Engineering at Harvard University
Harvard SEAS

Recent protocols in developmental biology are unlocking the potential for stem cells to undergo differentiation and self-assembly to form "mini-organs", known as organoids. To bridge the gap from organoid building blocks (OBBs) to therapeutic functional tissues, integrative approaches that combine bottom-up organoid assembly with top-down bioprinting are needed. While it is difficult, if not impossible, to imagine how either organoids or bioprinting alone would fully replicate the complex multiscale features required for organ-specific function – their combination may provide an enabling foundation for de novo tissue manufacturing. My talk will begin by describing our recent efforts to generate microvascularized organoids *in vitro* that exhibit enhanced maturation and function. Next, I will describe the generation of 3D vascularized organ-specific tissues by assembling OBBs into living matrices that support embedded printing of macro-vessels by a process known as sacrificial writing in functional tissue (SWIFT). Though broadly applicable, I will highlight our recent work on kidney, cerebral, and cardiac tissue engineering.

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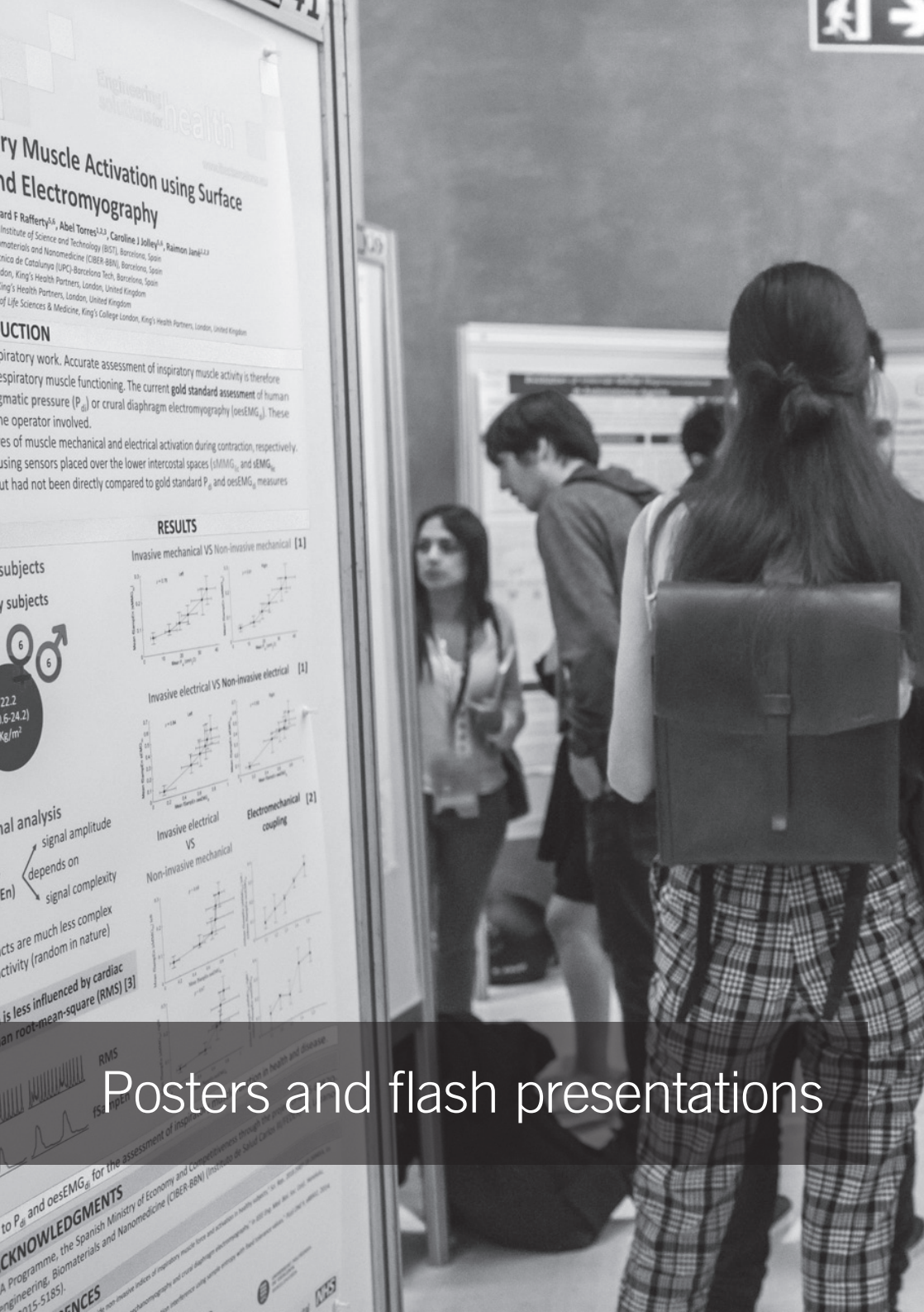
ONR NSSEFF (N000141612823)
Wyss Institute Organ Engineering Initiative

Jennifer A. Lewis

Harvard University

Jennifer A. Lewis *is the Jianming Yu Professor of Arts and Sciences, the Wyss Professor for Biologically Inspired Engineering in the Paulson School of Engineering and Applied Sciences, and a core faculty member of the Wyss Institute at Harvard University. Her research focuses on 3D printing of functional, structural, and biological materials that emulate natural systems. Prior to joining Harvard, Lewis was a faculty member in the Materials Science and Engineering Department at the University of Illinois at Urbana-Champaign, where she served as the Director of the Materials Research Laboratory. Currently, she directs the Harvard Materials Research Science and Engineering Center (MRSEC) and serves on the NSF Mathematical and Physical Sciences Advisory Committee.*

Lewis has received numerous awards, including the Presidential Faculty Fellow Award, the American Chemical Society Langmuir Lecture Award, the Materials Research Society Medal Award, the American Ceramic Society Sosman and Roy Lecture Awards, and the Lush Science Prize. She is an elected member of the National Academy of Sciences, National Academy of Engineering, National Academy of Inventors, and the American Academy of Arts and Sciences. She has co-founded multiple companies that are commercializing technology from her lab.



Posters and flash presentations

ICT FOR HEALTH - Posters with flash presentation

Poster	Name	Title
1	Yolanda Castillo-Escario	Electromyographic and Accelerometric Evaluation of Trunk Function and the StartReact Effect in Patients with Spinal Cord Injury

ICT FOR HEALTH - Posters

Poster	Name	Title
2	Dolores Blanco-Almazán	Analysis of Heart Rate Recovery after a 6-min walk test in Chronic Obstructive Pulmonary Disease Patients
3	Clare M. Davidson	Acoustic Features of Cough in COVID-19
4	Luis Estrada-Petrocelli	Inspiratory muscle activation evaluation in COPD patients with comorbid heart failure by means of surface mechanomyography
5	Adrián Fernández Amil	Information transfer of rate and phase codes in oscillatory neural networks
6	Ignasi Ferrer-Lluis	Sleep Apnea & Chronic Obstructive Pulmonary Disease: Polysomnographic Evaluation of Desaturations in Patients from an Epidemiological Study
7	Ismael Freire	Sequential Episodic Control
8	Manuel Lozano García	Noninvasive Assessment of Neuromechanical Coupling and Mechanical Efficiency of Parasternal Intercostal Muscle during Inspiratory Threshold Loading
9	Daniel Romero	Impact of Sleep Stages on Heart Rate Variability evaluated in Obstructive Sleep Apnea Patients
10	Vivek Sharma	BrainX3: A neuroinformatic tool for interactive exploration of multimodal brain datasets

NANOMEDICINE - Posters with flash presentation

Poster	Name	Title
11	Marc Azagra	Ammonium quantification (AQua) in human plasma by 1H-NMR for staging of liver fibrosis in fatty liver disease
12	Núria Blanco-Cabra	Dextran-Based Single-Chain Nanoparticles Improve the Tobramycin and DNase I Activity against Mature Biofilms by Interacting with the Extracellular Matrix
14	Madhura Murar	Peptide-based strategies for selective prostate cancer targeting using polymeric nanoparticles
15	Ignacio Viciano	In silico Identification of Potential Inhibitors of Sars-CoV-2 3CL Main Protease (MPro)
16	Lena Witzdam	Fight against thrombi: How can smart surfaces direct blood components to prevent thrombi
17	Xelia Ximenes-Carballo	Targeting wound healing with CaZn releasing platforms

NANOMEDICINE - Posters

Poster	Name	Title
18	Joana Admella	Monitoring Gene Expression during a *Galleria mellonella* Bacterial Infection
19	Julia Alcacer-Almansa	Pseudomonas aeruginosa and Burkholderia cenocepacia multispecies biofilms: an *in vitro* model for airway infections
20	Teodora Andrian	Correlating Super-Resolution and Electron Microscopy to Study Nanomedicine
21	Vetsy Verónica Arévalo-Jaimes	A new BiofilmChip device as a personalized solution for testing biofilm antibiotic resistance
22	Yunuen Avalos-Padilla	The role of Plasmodium falciparum ESCRT-III proteins in EV biogenesis and protein export
23	Manuel Bernabeu Lorenzo	Duplicated genes contribute to the virulence of pathogenic *E. coli* strains
24	Inés Bouzón-Arnáiz	Stimulation of protein aggregation in Plasmodium falciparum as antimalarial design strategy

25	Maria del Mar Cendra	*Pseudomonas aeruginosa* biofilms and their partners in crime
26	Manuela Garay-Sarmiento	Kill&Repel Coatings: the Marriage of Antifouling and Bactericidal Properties to Mitigate and Treat Wound Infections
27	Marina Inés Gianotti	Dissecting light- and redox-controlled Plastocyanin-Photosystem I interaction by single-molecule force spectroscopy
28	Larissa Hütter	Nernst-Planck-Poisson modelling of EGOFET biosensors
29	Mario Hüttener	Development of a novel vaccine to combat infections caused by multiple antibiotic resistant microorganisms
30	Rafael Ikemori	Lung cancer patient explant model for the direct assessment of drug responses
31	Christopher James	Fabrication and Characterisation of Lactate Releasing PLGA Particles
32	Maximilian Loeck	Glucocerebrosidase Deficiency Alters Plasmalemma Nano-Scale Domains and Transcytosis of Therapeutic Nanocarriers by Brain Endothelial Cells
33	Domingo Marchan del Pino	Unraveling the transcriptional regulation of the *nrdR* gene in *Escherichia coli* and *Pseudomonas aeruginosa*
34	Kate Neal	Investigating nanomechanical profiling as a novel diagnostic and treatment optimization approach with the ARTIDIS Platform: An example on two biological tissues
35	Marta Perxés Perich	Polyoxometalate-decorated gold nanoparticles as β-amyloid inhibitors.
36	Alejandro Prieto	Novel features of virulence regulation in enteroaggregative *E. coli*
37	Carlota Roca Martinez	Generation of DNA aptamers specific for enzymes of the methylerythritol pathway
38	Lucía Román-Álamo	Drug delivery nanovesicles for the treatment of leishmaniasis
39	Alba Rubio-Canalejas	Improving the treatment of a dual-species *S. aureus – P. aeruginosa* mixed wound biofilm: impact of antibiotic and enzymatic therapy, and 3D spatial organization
40	Noelia Ruiz Gonzalez	Motion of Enzyme-powered Nanomotors in Complex Media

41	Meritxell Serra-Casablancas	Enzymatic Nanomotors Towards Cancer Treatment
42	Shubham Tanwar	Exploring Different Operating Regimes of Electrolyte-Gated Organic Field-Effect Transistor at the Nanoscale
43	Eduard Torrents	Utility of *Galleria mellonella* larvae for evaluating nanoparticle toxicology
44	Morgane Valles	Upgrading the engine-chassis complex of micro-/nanomotors towards application-oriented designs
45	Guillem Vives	Optimizing Enzyme Encapsulation in Targeted Nanoparticles for Enzyme Replacement Therapy of Lysosomal Disorders
13	Ruben Millan-Solsona	Mapping the Nanoscale Dielectric Properties of Cells by Scanning Dielectric Force Volume Microscopy and Machine Learning

MECHANOBIOLOGY - Posters with flash presentation

Poster	Name	Title
46	Juan F. Abenza	Cell mechanics influences the mammalian circadian clock via YAP/TAZ
47	Giulia Fornabaio	Biomechanics of the progression of hypermethylated colorectal carcinomas
48	Ignasi Granero-Moya	Studying mechanotransduction in the nucleus: a multicellular approach

MECHANOBIOLOGY - Posters

Poster	Name	Title
49	Amy EM Beedle	Fibrillar adhesions confer mechanical memory to the nucleus
50	Gerardo Ceada	Mechanical compartmentalization of the intestinal organoid enables crypt folding and collective cell migration
51	Sefora Conti	Cancer stem cell and the metastatic cascade: a mechanical approach
52	Bárbara M. de Sousa	Capacitive electrical stimulation promotes neuritogenic signaling
53	Laura Faure	Cells exert pushing forces on their environment
54	Alejandro Llorente	Inhibition of the immunosuppressive effects of activated fibroblasts in non-small cell lung cancer
55	Ignasi Granero Moya	Development of a model of “macro” substrates for the analysis of 3D chromatin structure and transcriptional profiling
56	Macià Esteve Pallarès	A novel durotactic migration emerges from the proximity to a wetting transition
57	Srivatsava Viswanadha Venkata Naga Sai	A mechanobiological study of pluripotency dissolution in mouse embryonic stem cells

CELL ENGINEERING - Posters with flash presentation

Poster	Name	Title
58	Akinola Akinbote	Lung and Cardiac Microvascular Tissues on-Chip Reveals Differential Fibrotic Phenotypes
59	Pilar Alamán-Díez	Osteogenic differentiated adipose-derived stem cells create an *in vitro* bone model inside microfluidic platforms
60	Ignasi Casanellas	Dynamics of stem cell migration and condensation on nanopatterned adhesive ligands
61	Angela Cirulli	Photo-crosslinkable hydrogels for skin model reconstruction
62	Veronika Magdanz	Flexible Magnetic 3D Printed Robots for Cell and Drug Delivery
63	Marina Martínez-Hernández	Effect of lactate on cardiac fibroblast activation and its implications for in situ cardiac regeneration

CELL ENGINEERING - Posters

Poster	Name	Title
64	Valentina Serrano Cruz	Design of experiments to fabricate hydrogels by visible light photopolymerization using a 3D bioprinter
65	Gülsün Bağci	Cell-Derived Extracellular Matrices for Establishment of 3D *in vitro* Tumor Models for Cancer Research
66	Barbara Blanco Fernandez	Development of a bioprinted breast cancer model using tissue derived extracellular matrix
67	Víctor Campo-Pérez	Easily applicable modifications to electroporation conditions improve the transformation efficiency rates for rough morphotypes of fast-growing mycobacteria
68	Laura Clua	Collagen-tannic Acid Spheroids For β–cell Encapsulation Fabricated By 3D Bioprinting
69	Francesco De Chiara	Biomaterials for retina epithelial cell transplantation as treatment for degenerative macular disease

70	Natalia Díaz-Valdivia	Stromal SMAD3 enhances cancer cell invasion led by fibroblasts in 3D culture models
71	Ainhoa Ferret-Miñana	Fatty hepatocytes induce skeletal muscle atrophy in vitro: a new 3D platform to study the protective effect of albumin in Non-Alcoholic Fatty Liver Disease
72	Judith Fuentes	Skeletal muscle-based 3D-bioengineered actuators as platforms of biomedical interest
73	Maria Gallo	Generation of reporter human pluripotent stem cell lines to study cardiac and renal development and disease
74	Rosalina Gavin Marin	Study of epigenetic modifications related to the cellular prion protein expression as preclinical biomarkers of Alzheimer's diseas
75	Maria Guix	3D Engineered living systems as swimming robots
76	Alba Herrero Gómez	Cryopreservation of 3D cell culture models
77	Adrián López Canosa	Organ-on-a-chip System to Study Biomaterial-induced Vasculogenesi
78	Sara Martínez-Torres	Cannabinoid 1 receptor stimulation increases the regenerative capacity of sensory neurons
79	Mehrnoush Rahimzadeh	Study and analysis of class Ia ribonucleotide reductase from *Pseudomonas aeruginosa*
80	Francina Mesquida-Veny	Neuronal activity effects on axonal growth after spinal cord injury
81	Maria Narciso	Novel Method for the Quantification of Tissue Decellularization
82	Zarina Nauryzgaliyeva	Personalized Bioactive and Biodegradable Implants for Maxillofacial Bone Regeneration
83	Ana Pascual	3D Brain-on-a-chip for Organoptypic Culture and Differentiation of Neuroprogenitor Cells
84	Sergi Rey-Vinolas	Personalized Biodegradable 3D Printed Implant for Guided Bone Regeneration
85	Ines Sousa Pereira	Gelatin-based hydrogels compatible with neuronal culture and differentiation
86	Karen Isabel Wells Cembrano	Establishment of an *in vitro* biomimetic model for the study of human neuromuscular diseases

Electromyographic and Accelerometric Evaluation of Trunk Function and the StartReact Effect in Patients with Spinal Cord Injury

Yolanda Castillo-Escario[1,2,3], Hatice Kumru[4,5,6], Josep Valls-Solé[7], Loreto García-Alen[4], Joan Vidal[4,5,6], Raimon Jané[1,2,3]

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Spinal cord injury (SCI) causes impairment of motor and sensory functions below the injury and many other health complications. A common consequence of SCI is the lack of control over trunk muscles. This constitutes a major cause of motor disability, limiting patients' independence and quality of life, since trunk stability is essential for postural control, but also to support functional limb movements. However, trunk stability is rarely examined in studies of mobility after SCI, and one of the reasons is the lack of quantitative measures for assessing trunk function. In a previous study, we investigated trunk flexion during a reaching task in healthy subjects [1]. Here, we aimed to examine this task in SCI patients [2]. The objectives of this study were 1) to evaluate trunk muscle activity and movement patterns during a reaching task in SCI patients, from the analysis of electromyography (EMG) and smartphone accelerometry signals, and 2) to investigate the effects of a startling acoustic stimulus (SAS) in these patients.

EMG and smartphone accelerometer data were recorded from 15 patients with cervical SCI (cSCI), 9 patients with thoracic SCI (tSCI), and 24 healthy gender- and age-matched controls, during a reaching task requiring trunk tilting (Fig. 1). We calculated the response time (RT) until pressing a target button, EMG onset latencies and amplitudes, and trunk tilt, lateral deviation, and other movement features from accelerometry. After that, statistical analysis was applied to analyze the effects of group (cSCI, tSCI, control) and condition (SAS, non-SAS) in each outcome measure.

SCI individuals, and especially those with cSCI, presented significantly longer RT (Fig. 2) and EMG onset latencies than controls. Moreover, in SCI patients, forward trunk tilt was accompanied by significant lateral deviation, possibly as a compensatory strategy to improve sitting balance. On the other hand, RT and EMG latencies were remarkably shortened by the SAS (the so-called StartReact effect) in tSCI and controls, but not in cSCI patients (Fig. 2), suggesting an increased cortical control exerted by these patients to compensate for their deficits in postural control.

The outcome measures proposed in this study revealed motor changes and compensatory postural strategies employed by SCI patients, including delayed responses and higher lateral deviations, which might have important consequences for rehabilitation. The combination of EMG and smartphone accelerometer data can provide quantitative measures for the assessment of trunk function in SCI and clinical information relevant to the management of these patients.

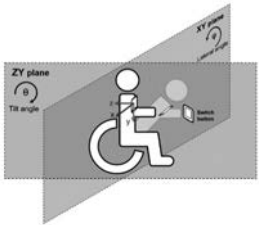

Figure 1. Representation of the task: the subject flexes the trunk forward to reach the switch button. A smartphone is placed at the subject's chest to record accelerometer data and derive the trunk tilt and lateral angles (*figure extracted from [2]*).

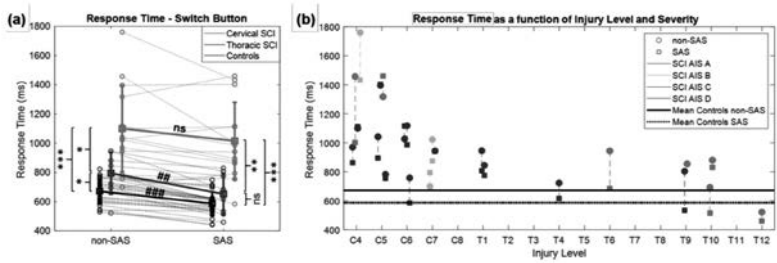

Figure 2. Response time (RT) for each group, in non-SAS and SAS trials (a), and RT of each SCI patient in non-SAS (circle) and SAS (square) condition, as a function of the injury level and severity (b). Asterisks and hashes indicate statistical differences between groups and between non-SAS and SAS trials, respectively (* p<0.05, ** p<0.01, *** p<0.001) (*figure extracted from [2]*)

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Analysis of Heart Rate Recovery after a 6-min walk test in Chronic Obstructive Pulmonary Disease Patients

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Patients with chronic obstructive pulmonary disease (COPD) exhibit impaired autonomic control, assessed by heart rate variability (HRV) analysis [1]. However, it is unknown whether dynamic markers from the heart rate time-series may identify autonomic dysfunction linked with disease severity and respiratory function. This study evaluated the cardiac autonomic responses after completing a conventional walk challenge in these patients [2]. The study included forty-six COPD patients who performed a standard six-minute walk test (6MWT) [3]. The ECG signal (lead II) was acquired before, during, and after the test using a wearable research device. Inter-beat interval time-series (RR intervals) were evaluated and used to assess the heart rate (HR) dynamic during recovery. In particular, we computed the heart rete recovery (HRR) markers at standard timings (at first and second minute) but also every 5 s throughout recovery following the 6MWT. We evaluated the resulting HRR markers and the normalized HRR values by the maximum HR just after the walking period (nHRR) among patient severity groups classified according to GOLD guidelines [4]. The results showed different dynamic trends among the severity groups. We observed significantly larger nHRR in less severe COPD patients (n=23, GOLD={1,2}; nHRR1=14.8±7.5%, nHRR2=18.6±8.1%) compared to those with more disease severity (n=23, GOLD={3,4}; nHRR1=9.3±5.8%, p=0.002; and nHRR2= 13.7±6.7%, p=0.041). The largest differences among groups were observed around the first 30 s of the recovery phase (nHRR=10.8±6.6% vs nHRR=5.6±4% p=0.001). Our results showed a slower recovery for patients who had worse diagnoses, probably due to a weaker response of vagal control reactivation. This suggests that, besides respiratory markers, cardiac parameters may provide valuable information for a better characterization of disease severity in COPD patients.

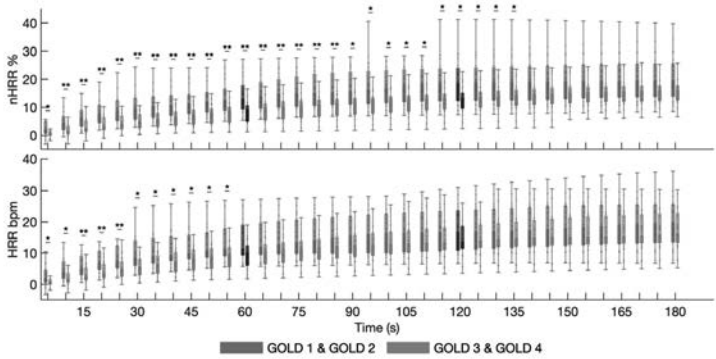

Figure 1 Evolution of the heart rate recovery, normalized by the maximum HR at the end of the test (nHRR) and the absolute value (HRR). The significant differences were analyzed by the Kruskal-Wallis test with Bonferroni's correction in the pairwise p-values. * denotes p-value < 0.05 and ** denotes p-value < 0.01.

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Acoustic Features of Cough in COVID-19

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Respiratory illness is experienced by many patients infected with SARS-CoV-2 (COVID-19), with a dry cough one of the most common symptoms. Cough sounds provide important information about respiratory function in both healthy and patient populations, and are often used in the detection, assessment, and monitoring of respiratory disease.

This study analyzes differences in cough signals recorded using a smartphone [1] in 24 patients hospitalized with COVID-19 at different disease stages. Cough recordings of patients in group A (N=12, 3 female, 53 (47-65) yrs.) occur at the most severe stage of the disease, and recordings of patients in group B (N=12, 3 female, 53 (46-63) yrs.) occur prior to the deterioration of the disease. Cough sounds were recorded at 48 kHz using the built-in microphone of a smartphone. Individual cough signals were manually segmented into 3 parts: the first cough sound, the intermediate part and the second cough sound (if present) [2]. For each segment, and the signal as a whole, the percentage power in each 250 Hz band was calculated and several frequency- based features were examined.

Three-way ANOVA (factors: disease status, gender, age group) revealed significant differences between the frequency variability in the first cough sound ($F=5.27$, $p=0.034$) for disease status, with the mean value of group A higher than B (670.77±193.72 Hz vs 512.24±140.82 Hz). The percentage power in each of three bands was significantly higher in group A compared to B also: 0-250 Hz ($F=6.13$, $p=0.024$), 1000-1250 Hz ($F=5.93$, $p=0.026$) and 3750-4000 Hz ($F=6.97$, $p=0.016$). Interaction effects of disease status, gender and age group were non-significant. The peak frequency of the intermediate part of the cough sound was significantly higher in female patients compared to male patients ($F=7.38$, $p=0.014$), as well as some features of the second cough sound: the median frequency ($F=4.99$, $p=0.04$) and the frequency at both the 25th percentile of signal energy ($F=4.58$, $p=0.046$) and the 75th percentile of signal energy ($F=4.61$, $p=0.048$).

The results outlined suggest that there are differences in the acoustic characteristics of the cough signal recorded in COVID-19 patients at different disease stages. However, differences between male and female patients analyzed here appear to be unrelated to disease status, with no significant interaction effects revealed. This work suggests that further exploration of the prognostic potential of the cough sound recorded in patients with COVID-19 holds promise.

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Inspiratory muscle activation evaluation in COPD patients with comorbid heart failure by means of surface mechanomyography

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Chronic obstructive pulmonary disease (COPD) is characterized by a persistent inflammatory process on airways and lungs with progressive and irreversible airflow limitation, interfering with normal breathing. COPD coexists with comorbidities that have a negative impact on patient's quality of life. Cardiovascular diseases, including heart failure (HF) manifest the same respiratory symptoms as prevalent in COPD. Both COPD and HF lead to impaired respiratory mechanics that increase the load on the respiratory muscle pump. Respiratory electromyography is a technique for recording the electrical activity generated by respiratory muscles and contributes to measure of the imbalance between the load on the respiratory muscles and its ventilatory capacity. However, this measurement is invasive in nature, restricting its use to trained medical personnel in clinical settings. Surface electromyogram of the lower intercostals ($sEMG_{IIC}$) muscles is a more accessible measure, facilitating the recording of respiratory muscle activity and integrates the contribution of the lower external intercostal muscles and costal diaphragm [1]. Surface mechanomyogram of the lower intercostal ($sMMG_{IIC}$) muscles is a novel measure of the mechanical response of the lateral oscillations of contracting respiratory muscle fibres.

The aim of this study was to investigate the respiratory muscle activation as evaluated by the $sEMG_{IIC}$ and $sMMG_{IIC}$ in COPD patients with and without comorbid HF (COPD-HF). Twelve COPD (10 male, 67.3±9.1 years, 27.3±4.2 kg/m2, FEV1 50.9±15.8 % pred) and 8 COPD-HF (6 male, 73.5±8.0 years, 29.4±5.0 kg/m2, FEV1 51.9±8.5 %pred) participated. $sEMG_{IIC}$ and $sMMG_{IIC}$ were bilaterally recorded along with the inspiratory mouth pressure during an incremental inspiratory threshold loading protocol at 0 (quiet breathing), 12, 24 and 36 % of maximum inspiratory mouth pressure (PImax) reached. $sEMG_{IIC}$ and $sMMG_{IIC}$ were processed using the fixed sample entropy (fSE), a technique that reduce heart's electrical and mechanical interference [1], [2] (Figure 1). $sEMG_{IIC}$ and $sMMG_{IIC}$ increased in both patient groups as inspiratory load increased, with greater inspiratory muscle activation in COPD-HF than in COPD patients [3] (Figure 2). Statistically significant differences were found between COPD and COPD-HF $sMMG_{IIC}$.

In conclusion, our study suggests that COPD patients with HF have greater respiratory muscle activity because of coexistence of both diseases compared to COPD patients. This work provides a novel way to investigate the inspiratory muscle activation in COPD patients with coexisting comorbidities, suggesting the potential value that combined electrical and mechanical non-invasive assessment of inspiratory muscles represents on the study of patients with respiratory disorders.

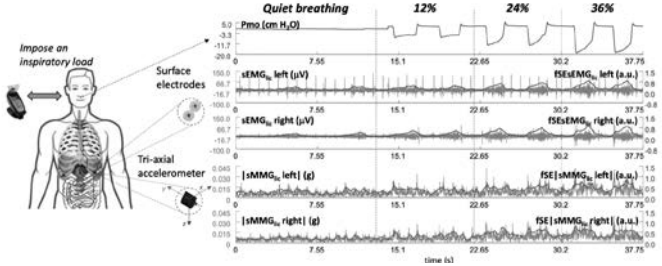

Figure 1. Incremental inspiratory threshold loading protocol. Left: Electrode set-up. Right: Traces represent the time-course of two respiratory breathings for inspiratory mouth pressure (Pmo) and the electrical and mechanical activity of the intercostal muscles (sEMGlic and sMMGlic) recorded on the lower left and right chest. sEMGlic and sMMGlic were expressed as the fixed sample entropy (fSE), (fSEsEMGlic and fSEIsMMGlicl) to reduce heart interference.

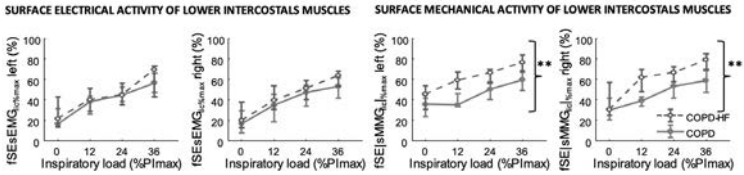

Figure 2. Evaluation of inspiratory muscle activation during an incremental inspiratory threshold loading (ITL) protocol as measured by the sEMGlic and sMMGlic activity on the lower left and right chest. sEMGlic and sMMGlic were expressed as the fixed sample entropy (fSE) and normalized to the maximum inspiratory pressure (fSEsEMGlic%max and fSEsMMGlic%max, respectively). During the ILT participants breathed unloaded at quiet tidal breathing (0%) and then the inspiratory load was adjusted to 12%, 24% and 36% of the maximum inspiratory pressure (PImax). Symbol denotes significant differences between COPD and COPD-HF set at p<0.01. The significance level was set at p<0.05.

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Information transfer of rate and phase codes in oscillatory neural networks

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The existence of place cells in the rodent hippocampus, together with their phase precession throughout the ongoing theta oscillation in the local field potential, suggests the coexistence of both a rate and a phase code to support spatial navigation. Yet, the properties of both neural codes have not been formally compared in the context of oscillatory dynamics. Here, by using an encoder-decoder framework representing the cortico-hippocampal circuit, we characterize two plausible coding schemes for information transmission: one based on spatially distributed firing rates, and another one based on phase-ordered spike sequences. Whereas the former transforms incoming analog input patterns into theta-confined firing rates, the latter recasts them into spike sequences in a theta-gamma phase format. Thus, while both codes rely on orthogonal ensembles firing throughout self-generated theta oscillations, they predict different -but physiologically plausible- readout mechanisms. Moreover, we show that, while both codes predict opposite distributions of the readout weights, they are equally learnable via STDP in a one-shot supervised learning scenario. Then, after matching both codes in terms of channel capacity, we show that whereas the rate code is more robust to input noise, the phase-order code is more energy-efficient, with the spikes per second scaling better with the number of ensembles. Finally, we highlight the relevance of our results for the coding of information and pattern recognition features of the mammalian hippocampus and the insect olfactory system.

Sleep Apnea & Chronic Obstructive Pulmonary Disease: Polysomnographic Evaluation of Desaturations in Patients from an Epidemiological Study

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Obstructive sleep apnea (OSA) is a sleep disorder in which repetitive upper airway obstructive events occur during sleep. These events can induce hypoxia, which is a risk factor for multiple cardiovascular and cerebrovascular diseases [1]. Chronic obstructive pulmonary disease (COPD) is a disorder which induces a persistent inflammation of the lungs. This condition produces hypoventilation, affecting the blood oxygenation, and leads to an increased risk of developing lung cancer and heart disease. The OSA-COPD overlap syndrome is the presence of both conditions at the same time, and it is expected to affect, at least,1% of the general population [2]. There have already been different attempts to study this overlap syndrome, which determined a variety of worsened consequences, such as the presence of hypoventilation during rapid eye movement (REM) sleep [3], the tendency of OSA-COPD patients to avoid sleeping in supine position [4] or the increased apnea hypopnea index (AHI) due to the increased occurrence of microarousals [5]. In this study, we evaluated how COPD affects the severity and characteristics of OSA in a German multivariate demographic database from the region of Pomerania, which included polysomnographic signals. Multiple features were analyzed related to the sleep position, the prevalence and characteristics of apneas and hypopneas and the characteristics of oxygen saturation (SpO_2). Results showed SpO_2 subtle variations, such as the ones from Figure 1, where more desaturations which did not recover the basal SpO2 level in a two-minute time window (non-recovered desaturations) and increased time below a 90% SpO2 level. These subtle differences were, in most cases, non-significant according to the Mann-Whitney-Wilcoxon statistical test. Nevertheless, it is important to consider that no GOLD 4 subjects were included, for which a more significant variation would be expected. This idea is reinforced when understanding that the sleep tests are performed in a resting scenario where the COPD condition is not stressed, and, for this reason, less severe COPD subjects tend to perform more equally to the control group, whereas more severe subjects would be expected to report more differences. Finally, it is important to consider that these SpO_2 subtle variations, in the long term, could worsen the risk to suffer cardiovascular and cerebrovascular diseases [6].

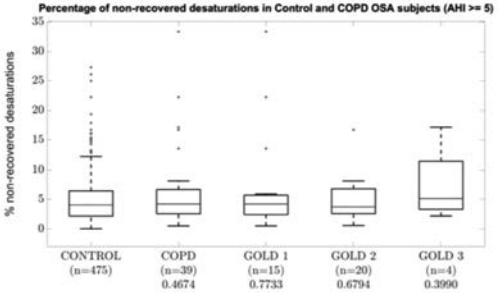

Figure 1. Boxplot of the percentage of non-recovered desaturations in non-healthy OSA subjects (AHI>=5) separated by five categories: Control, COPD, GOLD 1, GOLD 2 and GOLD 3. For each category, the number of subjects available (n) is provided.

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Sequential Episodic Control

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State of the art deep reinforcement learning algorithms are sample inefficient due to the large number of episodes they require to achieve their performance. Episodic Reinforcement Learning (ERL) algorithms, inspired by the mammalian hippocampus, typically use extended memory systems to bootstrap learning from past events to overcome this sample-inefficiency problem. However, such memory augmentations are often used as mere buffers from which isolated past experiences are drawn to learn from in an offline fashion (e.g., replay). Here we demonstrate that including a bias derived from the order of episodic sampling in the acquired memory content improves both sample and memory efficiency of an episodic control algorithm. We test our Sequential Episodic Control (SEC) model in a challenging foraging task to show that storing and using integrated episodes as event sequences leads to faster learning with less memory requirements as opposed to a control model that buffers isolated events only. Furthermore, we link our approach to the transition of deliberate-to-habitual behavior in mammals, showing how hippocampal-like fast episodic learning with cortical deliberation can bootstrap striatal-like slow habit learning to serve adaptive behavior.

Noninvasive Assessment of Neuromechanical Coupling and Mechanical Efficiency of Parasternal Intercostal Muscle during Inspiratory Threshold Loading

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Diaphragm neuromechanical coupling (NMC) is the efficiency of conversion of neural activation to transdiaphragmatic pressure, 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 transdiaphragmatic pressure and crural diaphragm electromyography, which complicates the measurement of diaphragm NMC in clinical practice.

We aimed to investigate, for the first time, noninvasive indices of NMC and mechanical efficiency (MEff) of parasternal intercostal muscles, calculated using surface mechanomyography ($sMMG_{para}$) and electromyography ($sEMG_{para}$) recordings [1]. The use of $sMMG_{para}$ as an inspiratory muscle mechanical output measure, and the relationships between $sMMG_{para}$, $sEMG_{para}$, and simultaneous invasive and noninvasive pressure measurements have not previously been evaluated. 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). $sEMG_{para}$, $sMMG_{para}$, and both invasive and noninvasive measurements of pressures were recorded in 12 healthy subjects during an inspiratory loading protocol. Myographic signals were analyzed using fixed sample entropy (fSampEn), which is less influenced by cardiac artefacts than conventional root mean square.

The ratios of $sMMG_{para}$ to $sEMG_{para}$, which provided muscle-specific noninvasive NMC indices of parasternal intercostal muscles, showed nonsignificant changes with increasing load (Figure 1b), since the relationships between $sMMG_{para}$ and $sEMG_{para}$ were linear (R2 = 0.85 (0.75–0.9)) (Figure 1a). The ratios of mouth pressure (P_{mo}) to $sEMG_{para}$ and $sMMG_{para}$ were also proposed as noninvasive indices of parasternal intercostal muscle NMC and MEff, respectively. These indices, similar to the analogous indices calculated using invasive transdiaphragmatic and esophageal pressures, showed nonsignificant changes during threshold loading (Figure 1d and 1f), since the relationships between P_{mo} and both $sEMG_{para}$ (R^2 = 0.84 (0.77–0.93)) (Figure 1c) and $sMMG_{para}$ (R^2 = 0.89 (0.85–0.91)) (Figure 1e) were linear. These results are equivalent to those found in our previous study using recordings from the lower chest wall inspiratory muscles

[2]. However, parasternal intercostal recordings have the advantage, over lower chest wall inspiratory muscle recordings, of being easier to acquire, being less influenced by chest wall thickness and subcutaneous fat, and being less susceptible to crosstalk from postural chest wall and abdominal muscle activity.

Our findings suggest that the proposed noninvasive NMC and MEff indices of parasternal intercostal muscles would therefore make the evaluation of respiratory muscle function easier and faster to perform, and thus more acceptable in patients with altered respiratory mechanics.

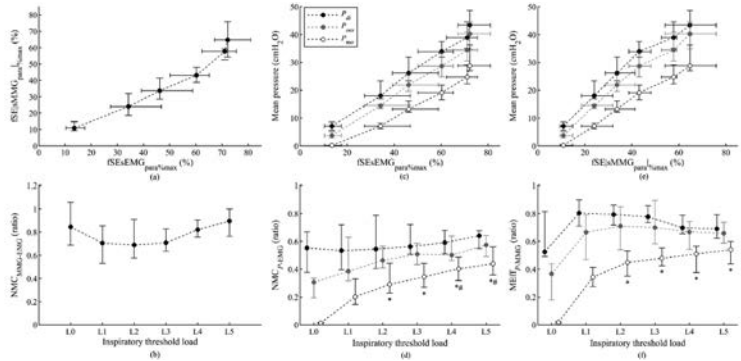

Figure 1. Relationship between fixed sample entropy measurements of surface mechanomyography ($fSEIsMMG_{paral\%max}$) and surface electromyography ($fSEsEMG_{para\%max}$) of the parasternal intercostal muscles (a) and the corresponding neuromechanical coupling ratio ($NMC_{MMG\text{-}EMG}$) (b), during inspiratory threshold loading. Relationships between respiratory pressures and both $fSEsEMG_{para\%max}$ (c) and $fSEIsMMG_{paral\%max}$ (e) measurements, and the corresponding neuromechanical coupling ($NMC_{P\text{-}EMG}$) (d) and mechanical efficiency ($MEff_{P\text{-}MMG}$) (f) ratios, during inspiratory threshold loading. Data points represent median and interquartile range of the study subjects for each load. Symbols * and # indicate statistically significant differences with respect to loads L1 and L2, respectively.

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Impact of Sleep Stages on Heart Rate Variability evaluated in Obstructive Sleep Apnea Patients

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Patients suffering from obstructive sleep apnea (OSA) usually present an elevated sympathetic tone of autonomic control caused by the intermittent hypoxia (IH) [1, 2]. In this study, we have evaluated the relationship between different sleep stages and major apnea characteristics such as duration, frequency, and event type. Moreover, the impact of apnea events on heart rate variability (HRV) spectral markers was assessed in different groups of disease severity. The hypnogram signal and the R-R interval time-series were extracted in 81 OSA patients from night polysomnographic (PSG) recordings [3]. The apnea-hypopnea index (AHI), defined as the gold-standard diagnostic index, was used to classify the patients as mild-moderate (AHI<=30, n=44) or severe (AHI>30, n=37). The normalized power of the very-low frequency (VLF: 0.003-0.04 Hz), low frequency (LF: 0.04-0.15 Hz), and high frequency (HF: 0.15-0.4 Hz) bands of RR series were estimated by a time-frequency method [4], and averaged for 1-min epochs, classified either as normal or apneic segments. The autonomic response and the impact of sleep stages were assessed in both segments to compare patient groups [5]. Deeper sleep stages (S1 and particularly S2) concentrated the shorter and mild apnea episodes (from 10 to 40 s) compared to lighter (SWS) and REM sleep stages, as illustrated in Fig 1. This pattern was even more pronounced for the group of severe patients. On the other hand, episodes longer than 50 s, although less frequent, presented similar incidence in all stages. Regarding HRV analysis, during apnea segments, LFnu was higher ($p=0.044$) for the severe group, since LFnu and HFnu presented the greatest changes when compared to normal segments. The non-REM sleep stages seems to better differentiate OSA patients' groups, particularly through LFnu and HFnu ($p<0.001$) markers (see Fig. 2). A significant difference in both sympathetic and vagal modulation between REM and non-REM sleep was only found within the group of more sever patients. Our results confirm the importance of considering sleep stages for HRV analysis to further assess OSA disease severity, beyond the traditional and clinically limited AHI values.

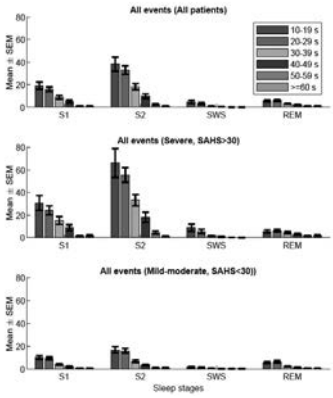

Fig 1. Average number (Mean ± SEM) of respiratory events (Apneas and hypopneas) per sleep stage evaluated for all patients (top), severe patients (middle), and mild-moderate patients (bottom). Colored bars represent duration ranges pooled in steps of 10 s.

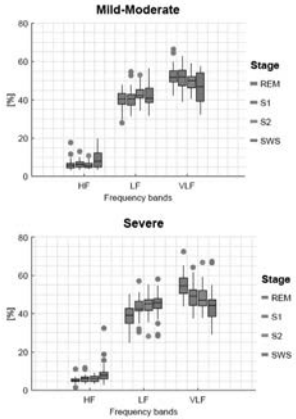

Fig. 2 Sleep-stage analysis and normalized HRV markers comparison for mild-moderate (left) and severe (right) patients during apneic epochs. REM: Rapid eyes movement; S1 and S2: deep sleep wave; SWS: slow sleep wave.

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BrainX3: A neuroinformatic tool for interactive exploration of multimodal brain datasets

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BrainX3 is a neuroinformatic tool for exploration, analysis, modelling and simulation of brain data using interactive visualizations that facilitates exploration and discovery of new insights by scientist and clinicians. We describe the design principles and architecture of the platform and the general features of the software. We present BrainX3 Radiology, an application built on top of the platform that targets the localization of brain lesions and the analysis of their impact on brain function for rapid and efficient access to high-quality integrated multi-modal brain data together with tools for analysis and semantic interpretation.

Ammonium quantification (AQua) in human plasma by 1H-NMR for staging of liver fibrosis in fatty liver disease

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Liver fibrosis staging is a key element driving prognosis of patients with chronic liver disease. Currently, biopsy is the only technique capable of diagnosing liver fibrosis in patients with alcohol- related liver disease (ArLD) and non-alcoholic fatty liver disease (NAFLD) unequivocally [1]. Although a direct relationship between fatty liver disease and high levels of ammonium (NH4+) in plasma has been demonstrated and explained by the downregulated activity of the urea cycle enzymes [2], non-invasive biomarker assays of ammonium in plasma must increase in reliability and sensitivity to be used clinically.

Here we demonstrate 1H nuclear magnetic resonance as a method to rapidly quantify endogenous concentrations of ammonium in human plasma extracts, showing ability to report upon advanced stages of ArLD and NAFLD (Figure 1). The procedure has been optimized testing different experimental conditions in order to achieve an accurate and realible quantification. Subject to validation in larger cohorts, the study indicates that the proposed method is likely to provide an accurate and rapid staging of ArLD and NAFLD without the need for an invasive biopsy.

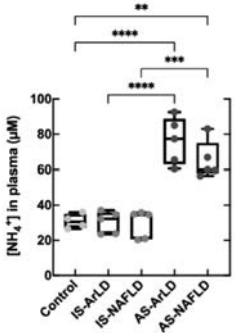

Figure 1: Concentration of ammonium present in plasma from healthy subjects (control; n=4), initial and advanced stages of alcohol-related fatty liver disease (IS- ArLD and AS-ArLD, respectively; n=5 for each group) and initial and advanced stages of non-alcoholic fatty liver disease (IS-NAFLD and AS-NAFLD, respectively; n=5 for each group). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

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Dextran-Based Single-Chain Nanoparticles Improve the Tobramycin and DNase I Activity against Mature Biofilms by Interacting with the Extracellular Matrix

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The extracellular matrix protects the biofilm cells by reducing the diffusion of the antimicrobials. Tobramycin is an antibiotic extensively used to treat P. aeruginosa biofilms, but due to its positive charges, it is sequestered in the biofilm periphery by the extracellular's negative charges matrix, losing efficacy significantly. Several nano-based formulations have been designed and are nowadays used to increase the final concentration of the antibiotics at the infection site, but alternatives to increment the diffusion of this antibiotic into the biofilm are necessary. Dispersal of the biofilm extracellular matrix with enzymes like DNase I is another used therapy against biofilms that can increment antibiotic penetration and diffusion to reach the internal bacterial cells. Here, we combine the charge neutralization effect that the dextran-based single-chain polymer nanoparticles (SCPNs) provide to the tobramycin with the aid of the DNase I to break the biofilm matrix. The effect of these SCPNs against continuous and mature P. aeruginosa and S. aureus biofilms is studied. Moreover, the specific interaction of SCPNs with the different extracellular matrix components of the P. aeruginosa biofilm was carefully analyzed, characterized, and their interactions were studied, showing an improvement of the activity of the tobramycin and the DNase I.

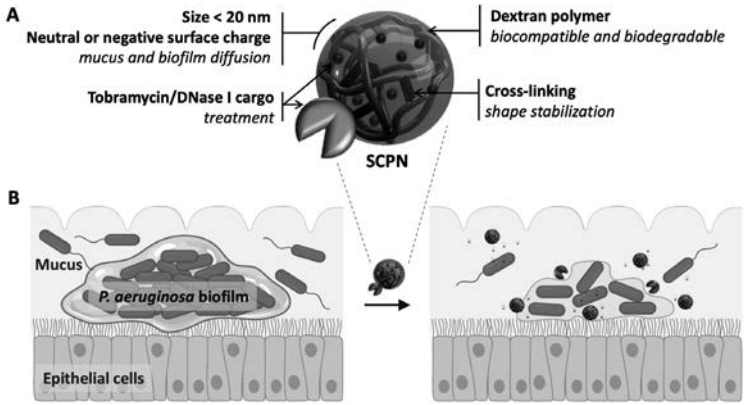

FIGURE: A) Rational design of multifunctional dextran-based single-chain polymer nanoparticle for treatment of chronic pulmonary infection. B) Illustration of biofilm degradation after treatment with the nanoformulation comprising tobramycin and DNase I.

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Peptide-based strategies for selective prostate cancer targeting using polymeric nanoparticles

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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. A key bottleneck of current cancer treatments is the lack of selective targeting of cancer cells to reduce undesirable side-effects. Nanoparticles (NPs) allow for the design of ligand-coated materials that can fulfil this function, however, have not shown consistent clinical results to make the theory of 'magic bullet' into a paradigm. These inconsistencies may arise from a multitude of biological factors like differences in disease models or expression levels of target receptor. NP design parameters could play a key role in alleviating these inconsistencies and significantly influencing the therapeutic efficacy. The properties of surface ligands such as their target receptor affinity, surface density, hydrophobicity etc. also play an important role in defining the targeting potential of a nanosystem.

Within this framework, we carry out a study of a cell targeting peptide (CTP) known as WQP peptide having a moderate/low affinity towards Prostate Specific Membrane Antigen, a known biomarker for prostate cancer (PCa) to evaluate the effect of its multivalency over monomeric form on cellular uptake. We find that by increasing the valency of WQP on the NP surface, we observe a higher cellular uptake of WQP-NPs over the monomeric form, which could attribute to a stronger avidity for selective PSMA targeting.

Additionally, we study the effect of two types of conjugation strategies using another CTP known as the GE11 peptide, which has a high affinity for Epithelial Growth Factor Receptor (another PCa biomarker), to obtain a specific GE11 density on NP surface and its impact on cellular uptake in a systematic manner. We observe that conjugating GE11 peptide to PLGA- PEG polymer prior to NP formulation (pre-conjugation) allows for a higher and more controlled GE11 density on NP surface than conjugating it to formulated PLGA-PEG NPs (post-conjugation), consequently leading to higher tumor cellular uptake. The knowledge obtained from these studies allow for an optimal design of dual NPs comprising both peptides in specific stoichiometric ratios, as a mean for a super-selective or personalized PCa targeting.

In silico Identification of Potential Inhibitors of Sars-CoV-2 3CL Main Protease (MPro)

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The novel coronavirus Sars-CoV-2 is responsible for Covid-19, a severe respiratory disease whose first cases were reported in the city of Wuhan (China) in November 2019. Since then, this disease has been spreading uncontrollably to become in a global pandemic, which has caused around 225 million infections and 4.63 million deaths worldwide (data estimated in September 2021).

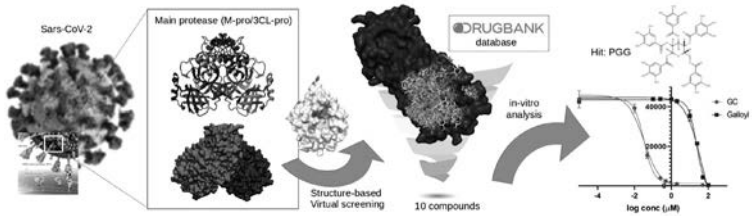
Apart from the health emergency, this disease is causing serious economic and mobility problems around the world, so that new antiviral treatments are urgently needed, in addition to the existing vaccines, to control the pandemic and avoid the appearance of new variants that could worsen it. Creating new drugs from scratch costs millions of dollars and it takes a long time for both development and clinical trials before they hit the market. The urgency of obtaining new drugs has made drug repositioning one of the tangible options for accelerated drug development in emerging diseases such as the Covid-19.

Several SARS-CoV-2 viral proteins have shown an important role in the replication of the virus, such as the SARS-CoV-2 Main protease (MPro or 3CL-pro), which is involved in the cleavage of polyproteins during the viral replication. Compounds capable of inhibiting these proteins could result in suitable antiviral candidates for the treatment of Covid-19 disease.

In the present study, we have carried out a structure-based virtual screening (VS) against the catalytic cleft of the Mpro enzyme using the DrugBank library of compounds, which is a database comprising around 10700 drugs classified as approved, investigational, experimental, withdrawn, nutraceuticals or even illicit.

The 38 most promising compounds from the virtual screening, consisting in a consensus docking strategy, were subjected to molecular dynamics (MD) simulations to evaluate the stability of the protein-small molecule binding mode. Subsequently, the free energy of binding was evaluated by means of MM-GBSA methods making use of the MD trajectories to predict the binding affinity more accurately.

After discarding some compounds that had been already reported in other studies or in clinical trials, we ordered 11 compounds, that we tested *in vitro* by means of a fluorogenic SARS-CoV-2 protease enzymatic assay. One of the 10 compounds tested *in vitro*, Beta-1,2,3,4,6-Penta-O- Galloyl-D-Glucopyranose (PGG), showed a 50% inhibitory concentration (IC50) of 24μM. PGG is a natural product that can be found in various fruits and natural plants.



Fight against thrombi: How can smart surfaces direct blood components to prevent thrombi

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The contact of blood with the surface of medical devices inevitably causes the activation of coagulation leading to a number of serious deleterious effects. As early as seconds to minutes after the contact of blood with the surface, protein adsorption occurs. This leads to the reciprocal activation of factor XII and plasma prekallikrein generating large amounts thrombin, which locally overwhelm the inhibitory effect of current anticoagulants. In indwelling devices as well as dialysis membranes or oxygenators the formation and dislodge of clots can lead to devastating problems as thrombosis, infarct and stroke. On the other hand, the lining of healthy endothelium is capable of sensing and maintaining a tightly regulated equilibrium, called hemostasis that prevents hemorrhages and excessive coagulation. Our goal is to develop coatings that turn the surface of medical devices hemocompatible to prolong their use without negative outcomes.

Towards this aim, our group develops coatings that inspired in the modulation mechanism of endothelium to go beyond passivation. The coatings are designed to interact with blood and orchestrate cascade of reactions to enhance their hemocompatibility and performance. They encompasses three hierarchical levels: a passive, an interactive and an adaptive one. The passive level consists of antifouling polymer brushes that create a physical barrier to protein adsorption and cell adhesion thereby prohibiting the surface-induced activation of coagulation. Here the goals are (i) to program repellency at nano and mesoscopic scales in the molecular structure of brushes and (ii) to develop methods to translate these finding to current medical devices.

The interactive level is achieved by decorating the brushes with biomolecules capable of binding to key elements of the coagulation cascade and inactivate them directly at the surface of the device. Compared to anticoagulants, this approach localizes the inhibitory effect at the surface and do not interfere with hemostasis. However, no coating, even natural endothelium is infallible. To address this we introduce an adaptive level. Here the goal is

that the coating sense the presence of a thrombus and orchestrate that its disintegration. To address this challenge, we develop a de novo fibrinolytic system that is only active in the presence of thrombus and orchestrate its destruction using components present in blood. After digestion, the coating returns to its dormant state.

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Targeting wound healing with CaZn releasing platforms

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Chronic wounds represent a major burden in human society, and the costs associated with its extensive care treatments are high; about 25 billion dollars are spent annually in the US[1]. These costs can be reduced by appropriate diagnose and treatment. Thus, research has focused 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 (Ca2+) and zinc (Zn2+) are essential for skin homeostasis. Calcium regulates platelet aggregation and epidermal stratification. We have shown that calcium-releasing platforms such as calcium phosphate nanoparticles (NPs) stimulate *in vitro* and *in vivo* wound healing [2,3]. On the other hand, deficiencies on Zinc are associated with impaired wound healing and roughened skin. Zinc's antimicrobial properties have been recently suggested [4,5], making this ion promising for its application on wound dressings.

This work aims to develop an ion releasing platform based on nanocomposites for local and sustained calcium and zinc release at the wound site, to promote wound healing.

Submicrometric particles incorporating different amounts of Zn2+ and Ca2+ ions were synthesized. Then, they were processed to modulate their degradability. Ion release was analyzed by colorimetric methods. pH was measured with a pH-Meter. Particle size was determined by Dynamic Light Scattering (DLS) and Scanning Electron Microscopy (SEM). Particle composition was studied using X-Ray Diffraction and Energy Dispersive X-Ray Spectroscopy (SEM-EDX). Direct and indirect particle toxicity was assessed *in vitro* in human dermal fibroblasts (hDFs) and dermal keratinocytes (hKCs) using the MTT assay. Wound healing potential was assessed using with the wound scratch assay.

Calcium and zinc were successfully incorporated into the particles in two different compositions. Particles showed a rounded morphology, with submicronic sizes. Ion release was sustained for up to 1 month (assay endpoint). Particles increased pH at the first 24h and then returned at physiological levels. These *In vitro* results indicate that, the lower the Zn content, the lower the toxicity. Particles stimulated cell migration and hKCs stratification.

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. On-going research to test their wound healing potential includes Matrix Metalloporteinase (MMP) and proangiogenic factor production, as well as collagen synthesis

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Monitoring Gene Expression during a *Galleria mellonella* Bacterial Infection

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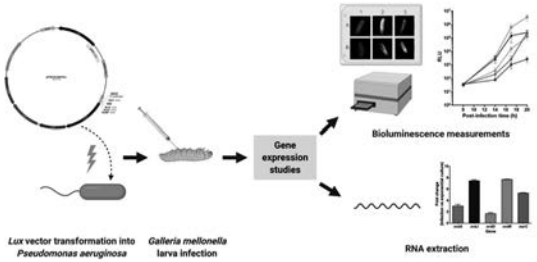
Galleria mellonella larvae are an alternative *in vivo* model that has been extensively used to study the virulence and pathogenicity of different bacteria due to its practicality and lack of ethical constraints. However, the larvae possess intrinsic autofluorescence that obstructs the use of fluorescent proteins to study bacterial infections, hence better methodologies are needed. Here, we report the construction of a promoter probe vector with bioluminescence expression as well as the optimization of a total bacterial RNA extraction protocol to enhance the monitoring of *in vivo* infections. By employing the vector to construct different gene promoter fusions, variable gene expression levels were efficiently measured in *G. mellonella* larvae at various time points during the course of infection and without much manipulation of the larvae. Additionally, our optimized RNA extraction protocol facilitates the study of transcriptional gene levels during an *in vivo* infection. The proposed methodologies will greatly benefit bacterial infection studies as they can contribute to a better understanding of the *in vivo* infection processes and pathogen– mammalian host interactions.



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Pseudomonas aeruginosa and Burkholderia cenocepacia multispecies biofilms: an *in vitro* model for airway infections

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Multispecies biofilms are communities composed of different microorganisms embedded in an auto-synthesized polymeric matrix. This matrix allows potential inter-species interactions that play an important role in chronic infections. Pseudomonas aeruginosa and Burkholderia cenocepacia are two multidrug-resistant and biofilm-forming opportunistic pathogens often found in the lungs of cystic fibrosis patients. There is a gap in existing biofilm-related literature between *in vitro* single-species biofilm studies and extensive metagenomic studies. In this context, a stable and balanced *in vitro* model to study P. aeruginosa and B. cenocepacia multispecies biofilms has been built following three steps. First, both species were studied in planktonic cocultures, where P. aeruginosa appeared to dominate, and B. cenocepacia lost its viability after 24 h of incubation. Then, the effects of the initial inoculum concentration, the growth media conditions, and the incubation time were evaluated in *in vitro* static biofilms. Finally, the optimal conditions for static multispecies biofilms were tested in a microfluidics-based dynamic biofilm formation system. A stable and balanced multispecies biofilm was obtained: 60 % of the biomass corresponded to P. aeruginosa and 40 % corresponded to B. cenocepacia, and both species' viability was maintained after 72 h of incubation. Such a model will potentially shed light on the effects of one population over the other in chronic infections as well as to test novel antimicrobial therapies to combat P. aeruginosa and B. cenocepacia polymicrobial infections.

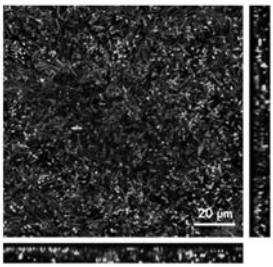

Figure 1. P. aeruginosa PAO1 and B. cenocepacia J2315 E2C multispecies biofilm in a dynamic system.

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Correlating Super-Resolution and Electron Microscopy to Study Nanomedicine

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The use of nanoparticles for the delivery of therapeutics (nanomedicine) is a promising research area, with a myriad of different formulations being developed[1,2]. Commonly, nanoparticles are decorated with ligands to target biomarkers of interest[3]. The interplay between size and ligand number and distribution is crucial for the formulation performance and need to be properly characterized to understand nanoparticle structure-activity relations. Yet, particle-to-particle heterogeneity poses a serious challenge due to the lack of methods able to measure both size and ligand distribution at the same time and at the single particle level[4]. Additionally, intracellular barriers such as endosomal entrapment can hinder the delivery of therapeutic cargo to the cytoplasm, reducing the effectiveness of various nanoformulations. The study and quantification of such processes is needed to ensure and predict the safety and efficacy of nanomaterials, but require methods with excellent resolution, molecular sensitivity, and cellular context, which cannot be offered by common ensemble techniques.

Correlative light and electron microscopy (CLEM) are a powerful and well-established group of multimodal imaging systems that merge the advantages of both microscopies through detailed images of the same region[5]. Super-resolution microscopy (SRM) is a more recent addition to the CLEM group[6], offering nanoscale resolution and unprecedented single-molecule localization precision[7]. Remarkably, despite the imaging potential of CLEM at the nanoscale, it has been mainly reserved for the field of biology and has not yet been applied for the structural characterisation of nanoparticles.

Here we address these gaps in nanomedicine by developing novel super-resolution (SRM) and transmission electron microscopy (TEM) correlative techniques. Firstly, we acquired SRM images of nanoparticles revealing the number and distribution of surface ligands with single-molecule sensitivity and TEM images for the same field-of-view showing the size and morphology at a with nanometric precision and at a single-particle level[8] (Figure 1). Secondly, we tracked nanoparticles intracellularly depicting their location within the endo-lysosomal pathway, paving the way for quantitative studies (Figure 2).

We believe and demonstrate that our CLEM techniques are applicable to a wide range of nanomaterials, and biological questions. The implementation of CLEM in nanoparticle characterization and intracellular tracking would allow the community to explore the relationship between various physicochemical properties and to better predict and understand their fate within the cell, bringing us a step closer to the successful design of nanomaterials.

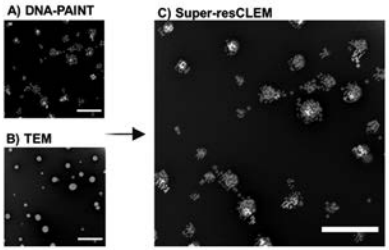

Figure 1. Correlative DNA-PAINT (a type of SRM technique) and TEM imaging of PLGA-PEG nanoparticles8. A) DNA-PAINT image where red localizations are representative of ligand number and yellow localizations of an encapsulated dye used as a reference marker. B) TEM image, corresponding to the same field-of-view as A. C) Overlaid CLEM image of A and B.

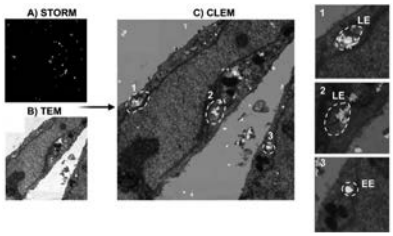

Figure 2. Correlative STORM (a type of SRM technique) and TEM imaging of PLGA-PEG after uptake by HeLa cells. A) STORM imaging where cyan signal is representative of single PLGA- PEG NPs encapsulated with fluorescent dye DiI. B) TEM image corresponding to the same field- of-view as A, depicting a HeLa cell. C) Overlaid CLEM image of A and B illustrating the position of various NPs within the cellular context. 1-3 zoom-in images from C showing the NP location in late endosomes and early endosomes.

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A new BiofilmChip device as a personalized solution for testing biofilm antibiotic resistance

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Currently, three major circumstances threaten the management of infections: increasing antimicrobial resistance, expansion of chronic biofilm-associated infections, and lack of an appropriate approach to treat them. To date, the development of accelerated drug susceptibility testing of biofilms and of new antibiofouling systems has not been achieved despite the availability of different methodologies. There is a need for easy-to-use methods of testing the antibiotic susceptibility of bacteria that form biofilms and for screening new possible antibiofilm strategies.

Here, we present a new easy method for testing antibiofilm susceptibility using a microfluidic device (BiofilmChip) that measures biofilm biomass by electrical impedance spectroscopy. BiofilmChip enables the growth of different bacterial species from clinically isolated strains and directly from sputum samples obtained from cystic fibrosis patients. Our results demonstrate that BiofilmChip is a useful tool for antimicrobial resistance testing in biofilms.

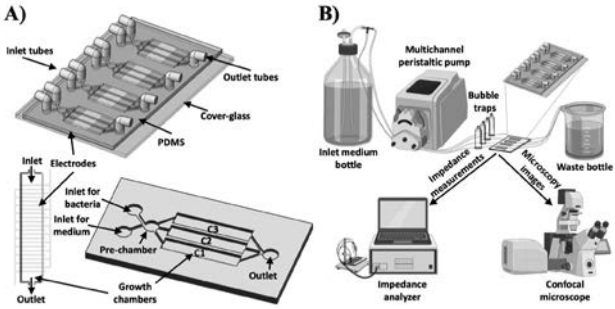

FIGURE: A) Biofilm chip design and B) experimental setup.

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The role of Plasmodium falciparum ESCRT-III proteins in EV biogenesis and protein export

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Infection with Plasmodium falciparum enhances the production of extracellular vesicles (EVs) in parasitized red blood cells (pRBCs) [1], an important mechanism for the pathophysiology of malaria disease. In higher eukaryotes there are two major EV biogenesis pathways: (1) Exosomes formation following the fusion of multivesicular bodies with the plasma membrane, and (2) microvesicle generation through plasma membrane shedding [2]. The Endosomal Sorting Complex Required for Transport (ESCRT) machinery has been implicated in both processes [3]. The core of the ESCRT machinery comprises ESCRT-I, -II, -III, ALIX/Bro1 and VPS4 sub-complexes. Among them, ESCRT-III is highly conserved across the eukaryotic lineage [4]. However, as Plasmodium lacks the upstream ESCRT complexes, the presence of a putative PfBro1 protein (whose counterpart in humans can directly recruit and activate ESCRT-III members [5]) was studied. A combination of different approaches revealed that P. falciparum uses a reduced ESCRT-III machinery to induce EV formation and contribute to protein export in pRBCs. We have demonstrated that PfBro1 activates either PfVps32 or PfVps60, both ESCRT-III members, which trigger EV biogenesis. The role of these proteins in membrane bud formation was reconstituted using giant unilamellar vesicles as a model system [6] in which we have visualized the assembly sequence and the function of the proteins. Moreover, we have detected the presence of all studied proteins in EVs secreted by P. falciparum-infected RBCs, demonstrating their participation in EV formation *in vivo*. Indeed, disruption of the Pfvps60 gene by CRISPR/Cas9 edition led to a significant reduction in the number of produced EVs and to significant changes in the proteomic profile of EV-exported proteins.

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Duplicated genes contribute to the virulence of pathogenic *E. coli* strains

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Pathogenic *E.coli* strains are grouped into pathotypes. Strains belonging to each pathotype show specific features, regarding both the virulence factors that they display and the host clinical symptoms. Enteroaggregative *Escherichia coli* (EAEC) are among the diarrheagenic *E. coli* pathotypes[1]. EAEC are the focus of active research because of their roles as etiologic agents of enteric infections that cause acute and persistent diarrhea in children and adults, persistent diarrhea in HIV-infected patients, traveler's diarrhea and extraintestinal infections such as urinary tract infections. The etiologic agent of an outbreak of foodborne hemorrhagic colitis in Germany in 2011 was an EAEC strain that displayed specific genomic features.

In a recent study, we were able to show that a feature of virulent *E. coli* strains is the presence of several duplicated genes that are absent in commensal strains. These genes can be specific either of virulent *E. coli* strains, of pathotypes and even of specific strains. Most of these genes are of unknown function. The fact that they are associated to virulent strains suggests that their gene products can contribute to the virulence.

In the present contribution we deleted a cluster of six genes that are present in four copies in the genomes of most of the virulent *E. coli* strains. We used for the study the EAEC strain 042. By different rounds of genetic manipulation, we deleted all four copies of the different genes selected. We compared thereafter the virulence of both the 042 wt strain and the deletion mutant by using the *Galleria mellonella* model. The results obtained show that the virulence of the deletion mutant is significantly reduced when compared to the parental strain.

Stimulation of protein aggregation in Plasmodium falciparum as antimalarial design strategy

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The Plasmodium falciparum proteome is clearly enriched in proteins with asparagine and glutamine repeats, which form low complexity regions (LCR) that have a propensity to form insoluble intracellular aggregates. An in depth in silico analysis found in this parasite 503 proteins containing domains capable of nucleating aggregation events. Moreover, aggregation in P. falciparum live cultures has been detected using an amyloid-specific stain.

Unregulated protein aggregation 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, 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. Actually, when the chaperone PfHsp110c was knocked down in P. falciparum, parasites were unable to prevent aggregation of LCR-containing proteins, leading to the pathogen's death.

P. falciparum proteostasis features could be useful in the design of radically new antimalarial strategies based on the potential toxicity for the parasite of an externally induced aggregation of its own proteome. To explore this hypothesis, we selected a set of aggregative peptides naturally present in P. falciparum parasites and delivered them to the pathogen using cell penetrating peptides and ghost erythrocytes as nanocarriers.

ACKNOWLEDGMENTS

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Pseudomonas aeruginosa biofilms and their partners in crime

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The ability of *Pseudomonas aeruginosa* to colonize medical devices and human tissues while growing in resistant communities called biofilms is a worldwide public health concern. P. aeruginosa biofilms have increased antibiotic tolerance and are more resistant to host responses than their planktonic counterparts, which makes the clearance of these biofilms difficult and infections chronic. A critical clinical trait of *P. aeruginosa* is its capacity to interact and coexist with other microorganisms in multispecies communities. From a clinical point of view, these interactions are usually detrimental to the patient, as infections caused by multiple species are often associated with worse prognosis. On the other hand, from a biotechnological perspective, there is a challenge to recreate the optimal conditions to grow multiple bacterial species simultaneously. *P. aeruginosa* can interact with other bacteria, fungi and viruses and together infect a wide range of human tissues (Figure 1).

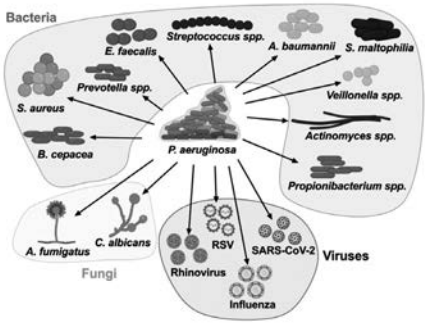

Figure 1. Scheme of the clinically relevant microbial biofilm interactions of *P. aeruginosa*. *P. aeruginosa* can interact and coexist simultaneously with a wide range of microbes from different phyla.

This study focuses on the main traits of *P. aeruginosa* biofilms, placing particular emphasis on the clinical challenges they represent in terms of antimicrobial susceptibility and biofilm infection clearance. Furthermore, it also highlights the main microbial interactions of Pseudomonas and the current models used to recreate them under laboratory conditions. The antimicrobial and antibiofilm strategies developed against *P. aeruginosa* mono and multispecies biofilms are also detailed.

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

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Even the most advanced antimicrobial dressings cannot fully protect and treat infected wounds because bacteria and their debris can adhere to the dressing and block the active antimicrobial surface. Moreover, dermal cells can attach causing adherence of the dressing to the wound and laceration during dressing exchange. Herein, we propose the Kill&Repel coating strategy, which is an ultra-thin coating that simultaneously kills bacteria and prevents adhesion of their debris and dermal cells.

The coating consists of a combination of polymer brushes and endolysin. The former provides antiadhesive and antifouling properties, while the latter hydrolyzes the peptidoglycan of the bacterium upon contact, causing its death. The formation of the coating on different types of dressings is achieved based on our new concept of utilizing the peptide, liquid chromatogryphy peak I (LCI), as an anchor of the polymer and endolysin to the surface. Poly(carboxybetaine methacrylamide) and poly(N- hydroxypropyl methacrylamide) were directly grafted from the LCI. The oriented adsorption of LCI on the surface segregates the polymer forming a 5-nm-thick brush–like coating.

The Kill&Repell coating prevented the adsorption of proteins as well as adhesion of fibroblasts and the pathogens E. coli and S. agalactiae, thereby turning the surface invisible to cells. Moreover, the synergistic action of the brush and the enzyme not only prevented the colonization of the dressing, but also enabled the coating to kill planktonic bacteria with even higher efficiency than the free enzyme. Remarkably, the Kill&Repel coating could completely eradicate bacteria in a simulated infection without allowing the adhesion of residues on the surface. Thus, this strategy opens a revolutionary approach for protecting and treating an infected wound in a safer yet more efficient manner.

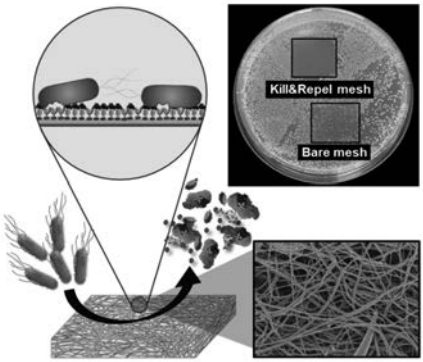

Figure 1. Kill&Repel coating: Coated PCL fibers prevented bacteria from adhering. In addition, the coated dressings were able to eliminate a simulated infection.

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Dissecting light- and redox-controlled Plastocyanin-Photosystem I interaction by single-molecule force spectroscopy

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Oxygenic photosynthesis is an efficient light- and redox-dependent process that converts light energy into chemical energy. One of the main steps in oxygenic photosynthesis is the link between integral membrane complexes present in the photosynthetic electron transport chain (PETC). Soluble redox proteins play a central role by connecting these integral membrane complexes that drive the electron transport process (ET). In plants, Plastocyanin (Pc) is a small water-soluble copper-containing redox protein responsible for shuttling electrons between Cytochrome *b6f* complex (Cyt*b6f*) and Photosystem I (PSI). While the effective encounter of Pc with PSI has been described to be determined by specific interactions (hydrophobic and electrostatic interactions), the effect of the redox states and light on the frequency of interaction and force of the PSI-Pc interaction is not clear. To date, the effect of the redox states of PSI and Pc in forming a productive complex has not been studied by atomic force microscopy (AFM).

This study work focuses on the characterization of the interaction process between well-oriented PSI (exposing the P700 luminal side towards the solution) and Pc (exposing the active site towards the solution) in different redox and lighting conditions using the AFM-Force Spectroscopy technique. PSI was immobilized via linker peptide on transparent gold electrode and Pc was immobilized to AFM probe via PEG_{27} linker. By lighting PSI and using reducing agents, we were able to control the redox states of PSI and Pc in situ and characterize the frequency and the force of the interaction associated with these different redox states. Our results indicate that the frequency of interaction between PSI and Pc depends on the biomolecules' redox states. On the other hand, the differences in the unbinding forces recorded in the different experimental conditions were related to how favorable the initial binding conditions of the Pc-PSI system are.

In addition, we also evaluate whether the presence of divalent cations (in different lighting and redox conditions) alters the frequency of interaction in the PSI-Pc system. We observe that the presence of Mg^{2+} decreases the frequency of interaction under reducing and lighting conditions. In plant chloroplast, Mg^{2+} concentration in the luminal side decreases when PSI is photoexcited. Our results suggests that the cellular control of Mg^{2+} levels by light could effectively and directly regulate the PSI-Pc interaction and therefore, the ET process.

Nernst-Planck-Poisson modelling of EGOFET biosensors

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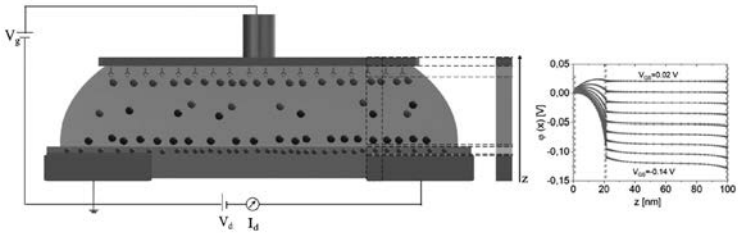
The need for accessible, low-cost, biocompatible and fast biosensing devices to detect diseases became more aware than ever. Electrolyte-gated organic field-effect transistors (EGOFETs) are being investigated for this purpose as they provide a highly sensitive recognition of various biomarkers[1] or are able to record bioelectric signals of medical relevance[2]. EGOFETs, as any field-effect transistor, are operated with three electrodes (source, drain and gate) and an electric current flows along an organic semiconductor material between the source and drain electrode modulated by the gate electrode. In the case of EGOFETs the dielectric separating the gate from the semiconductor material is an electrolyte, in which the ions accumulate at the interfaces with the gate electrode and the semiconductor channel (Fig. 1). The gate or semiconductor interfaces to the electrolyte can be functionalized (e.g. by attaching antibodies on the surfaces) and the device becomes a biosensor in which the electric current in the device becomes a direct response to the biorecognition event. The biorecognition event can implay a charge and/or capacitance modification of the interface, which modulates the ionic distribution in the electrolyte and hence the conductivity in the semiconductor material[3]. In the present work, we present the theoretical modelling of EGOFET biosensors described in the framework of the Nernst-Planck-Poisson theory of electrolytes and of the drift-diffusion theory of semiconductors. With this approach, we can predict the effects that charges and capacitance modifications on the recognition biolayer bring to the current flowing through the EGOFET. From the study we propose a generalized phenomenological model that allows a more accessible and faster analysis of experimental data than existing models. We show how experimental data corroborate some of the predictions of the theory.



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Development of a novel vaccine to combat infections caused by multiple antibiotic resistant microorganisms

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Bacterial infectious diseases, despite the availability of antibiotics, remain an important public health issue, representing the second leading cause of death worldwide. Horizontal transfer of bacterial plasmids generates genetic variability and contributes to the dissemination of the genes that enable bacterial cells to develop antimicrobial resistance (AMR). In our research group we have identified genes encoding high-molecular-weight proteins that contain one or several immunoglobulin-like domains (Big) and that are located in the transfer regions of several plasmids that usually harbor AMR determinants. These Big proteins are exported to the external medium and target two extracellular organelles: the flagella and the conjugative pili. The hitherto identified Big proteins are encoded in plasmids of different incompatibility groups: IncHI, IncA/C and IncP2. All of them are harbored by strains belonging to some of the most critical AMR microorganisms: *E. coli, Klebsiella, Citrobacter, Salmonella* and *Pseudomonas*. The fact that these proteins are plasmid-encoded and that they are targeted to the outer surfaces of the cell prompted us to consider that they can be immunogenic and targeted by the host immune system.

As a first approach, we have purified both the RSP and RSP2 proteins, encoded by IncHI plasmids, and used them as antigens to protect mice against infections caused by a Salmonella strain that is resistant to ampicillin due to the presence of IncHI plasmid pHCM1. In the present communication we show that, as predicted, mice that have been immunized with a mixture of the RSP and RSP2 proteins are protected against the infection caused by that strain.

Lung cancer patient explant model for the direct assessment of drug responses

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Lung cancer is the most common cause of cancer-related deaths worldwide and, despite the advance of new therapies, it displays a 5-year survival rate of only ~18% in developed countries, providing a large room for treatment improvement. To investigate new potential therapeutic compounds, there is a widespread use of cancer models that rely mainly in standard 2D cell cultures, which lack important stromal interactions provided by immune cells and tumor- associated fibroblasts. These cell types comprise the great majority of the tumor stroma and are decisive for the clinical outcomes, yet they are frequently not included when designing experimental tools for drug development. Seeking new instruments for drug screening and a better comprehension of their impact on the tumor microenvironment, our group has established an innovative patient explant model in Non-Small Cell Lung Cancer (NSCLC). Our model consists of using fresh human tumor samples, which are sliced and cultured in collagen-coated transwells, and then tested for drug screening. To validate our model, we have treated a NSCLC sample with Nintedanib, a multi kinase inhibitor and antifibrotic drug, which demonstrated antifibrotic effects in a time and dose-dependent manner that were consistent with clinical observations. Our encouraging preliminary results illustrate the exciting possibility of directly monitoring a diverse range of therapies for efficient drug screening, a crucial step for personalized medicine in the future.

Fabrication and Characterisation of Lactate Releasing PLGA Particles

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Lactate (or lactic acid) is now known to be utilised as a fuel and as a regulator in a range of cellular processes including immune tolerance, memory function, ischaemic tissue injury, cancer growth, metastasis, and wound healing.[1] Systemic or local delivery of lactate in mice with ischemic wounds was shown to increase reparative angiogenesis through endothelial progenitor cell recruitment and increased deposition of extracellular matrix. This led to accelerated wound healing and reduced skeletal muscle atrophy.[2] [3]Recent work by our group also showed that lactate promoted neuronal stem cell/progenitor maintenance and cardiomyocyte dedifferentiation (preprint).[4]

Utilising a well-defined synthesis method, water-oil-water emulsion, we fabricated poly(lactic-co- glycolic) (PLGA) particles loaded with lactate and characterised their properties such as size and lactate release. Tuning the polymer length and the ratio of lactic to glycolic acids within the PLGA used to synthesise particles, as well as the method for fabrication (e.g., polymer concentration, ultrasonication intensity and time) used has allowed tuning of their size and biodegradation rate, and so their lactate release profile.[5] The use of a cryoprotector was also investigated to prevent particle aggregation during the freeze-drying process.

Through this project, PLGA particles with sizes 200-2000 nm that release up to 1.6 mmol of lactate per mg in a week have been achieved, reaching biological active concentrations. The assessment of its efficacy in biomedical applications is currently under investigation.

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Glucocerebrosidase Deficiency Alters Plasmalemma Nano-Scale Domains and Transcytosis of Therapeutic Nanocarriers by Brain Endothelial Cells

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Deficiency of the lysosomal enzyme glucocerebrosidase (GBA) associates with neurological anomalies such as Gaucher and Parkinson's diseases. Treatments against these disorders remain scarce because the blood-brain barrier (BBB) prevents therapeutics from entering the brain. Drug nanocarriers (NCs) targeted to the blood-to-brain routes regulated by endothelial cells in brain capillaries may offer a treatment avenue. However, how GBA deficiency affects these routes is largely unknown. We examined this question using a pharmacological model to induce GBA deficiency in human brain endothelial cells, poly(lactide-co-glycolide) and polystyrene NCs, quantitative super-resolution STED microscopy for single-molecule tracking and radioisotopic tracers, to identify the most amenable trans-endothelial routes for drug delivery. GBA deficiency did not alter the nano-scale distribution of intercellular adhesion molecule 1 (ICAM-1), transferrin receptor (TfR), and plasmalemma vesicle-associated protein PV1, which associate with the CAM, clathrin, and caveolar routes, respectively.

Out of the three receptors, ICAM-1 nanodomains were most abundant, followed by TfR ones and then PV1. GBA deficiency slightly decreased the density of ICAM-1 nanoclusters and enhanced ICAM-1 lateral mobility, increased the levels and altered the nano-sale distribution of ganglioside GM1 and decreased caveolin-1 nanodomains. NCs targeted to these receptors all showed specific interaction with cells compared to non- targeted counterparts. GBA deficiency lowered NC binding, which may relate to the slightly decreased receptor cluster-density, the greater receptor lateral diffusion and/or altered lipid composition. NCs targeted to ICAM-1 bound more profusely to cells than TfR ones and both surpassed PV1 NCs, in agreement with their expression levels. Uptake of anti-ICAM NCs by brain endothelial cells was faster in GBA deficiency likely due to the enhanced confinement of ICAM-1 mobile fraction and increased immobile fraction observed, which may favor the formation of signaling platforms conducive to uptake. Over time, all NCs were transported across brain endothelial cells, yet GBA deficiency decreased transport to different extents depending on the targeted route, resulting in a greater number of anti-ICAM NCs being transported compared to NCs targeted to other routes.

This is the first time that the biological links between biochemical/biophysical membrane alterations, receptor mobility/nanoclustering, and transport across brain endothelial cells are studied, revealing key information about the biology of these diseases, and identifying the best routes to access the brain for their treatment.

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Unraveling the transcriptional regulation of the *nrdR* gene in *Escherichia coli* and *Pseudomonas aeruginosa*

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Ribonucleotide reductases (RNRs) are enzymes whose main function is to catalyze the conversion of ribonucleotides to deoxyribonucleotides (dNTPs), the precursors for DNA synthesis and repair. All living cells have, at least, one RNR encoded in the genome, but some organisms have more than one RNR class allowing them an advantage in different environments. Recent publications have described the transcriptional regulation of different RNR classes; however, little is known about how the expression of different RNRs might be coordinated in microorganisms encoding several RNR classes. A novel transcriptional factor, NrdR, has been implicated in the regulation of all three RNR classes through binding to conserved NrdR boxes in the promoter regions of almost all genes encoding RNRs. To better understand how NrdR regulates the different RNR genes, first, we need to understand how this gene is transcriptionally regulated.

Two organisms related to recent studies of NrdR are: Escherichia coli and Pseudomonas aeruginosa, each of which encodes three RNR classes (class Ia, Ib and III and Ia, II and III, respectively). In this work, a transcriptional regulation study of *nrdR* was made using bioinformatics and experimental tools. One of the first results was a different *nrdR* gene expression pattern in *E. coli* and *P. aeruginosa* during the bacteria growth cycle. Furthermore, different transcriptional regulation binding sites were identified in both bacteria's nrdR promoter region, showing a relationship with bacterial biofilm formation and cell cycle regulation. Our preliminary results bring us a step closer to the understanding of this unknown transcriptional regulation system.

ACKNOWLEDGEMENT

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Investigating nanomechanical profiling as a novel diagnostic and treatment optimization approach with the ARTIDIS Platform: An example on two biological tissues

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Lung cancer treatment has advanced significantly in the last 15/20 years with the advent of targeted therapies and immune checkpoint inhibitors. Still, only around 20% of patients will respond to immunotherapies, leaving the field wide open for the discovery of novel biomarkers to further stratify patients and prescribe the most suitable treatment. Moreover, as most lung cancer patients will only present at later stages of the disease (Stage III/IV), treatment options are limited and prognosis is poor, further highlighting the need to speed up the diagnostic process.

While there is a considerable focus on identifying genetic biomarkers, emerging evidence suggests that mechanical alterations inside the cell and in the extracellular matrix (ECM) is indicative of the metastatic/invasive potential of the cell. In addition, nanomechanical measurements are fast becoming a viable diagnostic companion to current molecular techniques. Atomic force microscopy (AFM) consists of a sharp nanometer scale tip attached to a flexible cantilever, which is scanned over the surface of a sample, and subsequently provides quantifiable topographical and mechanical information. ARTIDIS proprietary 20 nm AFM-based nanomechanical probe can provide a mechanical fingerprint of human tissue biopsies in under three hours, previously successfully differentiating malignant and benign breast cancers, and even stratifying them by subtype.

In the first clinical study of its type, we will analyze 75 non-small cell lung carcinoma (NSCLC) biopsies from Hospital Clínic using the ARTIDIS AFM, with the aim of obtaining

a mechanical profile which can differentiate tumor and healthy lung tissue and, when combined with clinical information, can further stratify patients according to the genetic and immune composition of their tumors. Not only would a shorter diagnostic time reduce patient anxiety, but it may also buy valuable time for treatment initiation. Additionally, with an accurate and personalized diagnosis according to the patient's specific tumor composition, the correct treatment can be selected first time, increasing the chance of successful treatment, and reducing burdensome over-treatment.

Polyoxometalate-decorated gold nanoparticles as β-amyloid inhibitors

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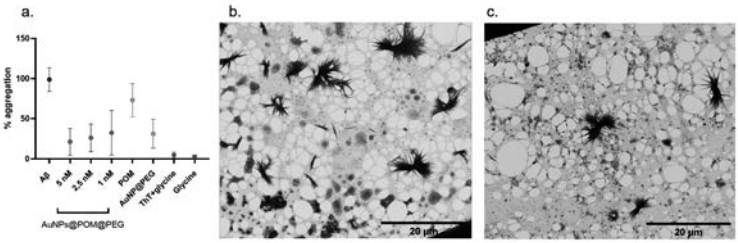
Alzehimer's disease (AD) is characterized by progressive neurodegeneration, being loss of memory the main symptom. One of the two main hallmarks is the amyloid plaque, formed by extracellular aggregates of amyloid fibrils [1,2]. Those are associated with the β-amyloid protein, that can polymerize forming aggregates, resulting in disfunction of axons and dendrites[3]. β- amyloid aggregation has been thoroughly studied, and numerous ligands have been synthesized in order to target it and to inhibit its aggregation[4], such as the new treatment recently approved by the FDA[5].

Polyoxometalates (POMs) are early transition metal oxide inorganic anionic clusters very well known for their redox activity[6]. Its negative charge allows them to bind to the β-amyloid monomer, which lowers the concentration of free monomer and shifts the equilibrium away from fibrillization[7]. Therefore, POM-decorated gold nanoparticles (AuNPs@POM) could be used as a drug delivery system for inhibiting β-amyloid fibrillization[8,9].

In the framework of this project, gold nanospheres have been functionalized with POMs, specifically with β_2 isomer of the monolacunary 11-tungstosilicate (β_2-$K_8SiW_{11}O_{39}$), which has promising effects inβ-amyloid fibrillization inhibition. To evaluate the system as a possible therapeutic agent for β-amyloid fibrillization inhibition, AuNPs@POM *in vitro* assays to assess cytotoxicity in neurovascular cell cultures were performed. Also, β-amyloid fibrillization inhibition *in vitro* by AuNPs@POM was evaluated as well as the permeability of AuNPs@POM through the blood brain barrier (BBB) in an organ on a chip model.
We found AuNPs@POM to be promising for β-amyloid aggregation inhibition, because they are not cytotoxic below 2.5nM concentration, they decrease β-amyloid aggregation *in vitro* and they can cross the BBB in the organ on a chip model without further modification.



β-amyloid aggregation inhibition by AuNPs@POM@PEG. a. % of aggregation respect to Aβ monomer alone of different conditions (n=6) b. STEM image at 20 kV of Aβ fibrils after fibrillization without AuNPs@POM@PEG. c. STEM image at 20 kV of Aβ fibrils after incubation with AuNPs@POM@PEG 1 nM.

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Novel features of virulence regulation in enteroaggregative *E. coli*

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Enteroaggregative *Escherichia coli* (EAEC) are among the diarrheagenic *E. coli* pathotypes. EAEC are the focus of active research because of their roles as etiologic agents of enteric infections that cause acute and persistent diarrhea in children and adults, persistent diarrhea in HIV-infected patients, traveler's diarrhea and extraintestinal infections such as urinary tract infections. The etiologic agent of an outbreak of foodborne hemorrhagic colitis in Germany in 2011 was an EAEC strain that displayed specific genomic features. A common genomic feature of most EAEC strains is the presence of a virulence plasmid termed pAA. pAA plasmids encode a transcriptional activator named AggR, which is a member of the AraC-XylS family of transcription factors.

When genetically manipulating the aggR gene in the EAEC strain 042, an unexpected phenotype was observed. Alterations in the 3'UTR of the gene result in clones showing enhanced expression of the *aggR* gene. In the present report we have analyzed the nature of the overexpression of the *aggR* gene in clones containing DNA insertions in the 3'UTR and show that *aggR* transcripts are extended far beyond the *aggR* gene. This results in the degradation of the *aggR* transcript. Alterations in the *aggR* 3'UTR alter transcript degradation and lead to AggR overexpression, which in turns induces the expression of some well-characterized virulence factors, such as the aggregative adherence fimbriae and specific toxins, and also of other, yet unreported genes, such as the *ast* and *fad* metabolic pathways. We provide evidence for these metabolic pathways contributing to the virulence of EAEC.

Generation of specific DNA aptamer for 1-deoxy-D-xylulose-5-phosphate reductoisomerase

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Pathogenic microorganisms such as the bacteria Mycobacterium tuberculosis and Pseudomonas aeruginosa, and the protozoa of the phylum Apicomplexa, including the causing agents of malaria and toxoplasmosis, synthesize the isoprenoid precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), by the methylerythritol phosphate (MEP) pathway. This pathway is essential for most bacteria and Apicomplexa, but it is not present in humans, which synthesize IPP and DMAPP by the alternative mevalonate pathway. The essential role of the MEP pathway and its distribution in different organisms make their enzymes attractive targets for the development of new anti-infective agents. Herein, we focus on the development of aptamers against key enzymes of the MEP pathway. Aptamers are single-stranded oligonucleotides which behave as "chemical antibodies" and can bind specifically and efficiently to a given target molecule. We report the identification of a DNA aptamer (D10) which specifically binds to the enzyme catalyzing the first committed step of the MEP pathway: 1-deoxy-D-xylulose-5-phosphate reductoisomerase. The aptamer was identified by systematic evolution of ligands by exponential enrichment, an in vitro selection process based on iterative cycles of binding, partitioning, and amplification of oligonucleotides from a pool of variant sequences. The results obtained suggest that the D10 DNA aptamer could be a potential candidate for the development of new therapeutic agents and for the design of novel diagnosis systems.

Keywords: aptamers, antibiotics, antimalarials, anti-infective agents, isoprenoids, methylerythritol pathway,

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Drug delivery nanovesicles for the treatment of leishmaniasis

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Leishmaniasis is a neglected tropical disease transmitted by sandflies and caused by the protozoan parasite *Leishmania*, of which more than 20 different species can infect humans. *Leishmania* has two morphological forms, termed amastigotes and promastigotes, found in mammalian and sandfly hosts, respectively. Depending on the parasite species and the immune response from the host, there are three different clinical manifestations of the disease: visceral (VL), cutaneous (CL), or mucocutaneous leishmaniasis (MCL). According to the World Health Organization (WHO) there are every year an estimated 30,000 new cases of VL and more than one million of CL (WHO, 2021). Because current medicines for treating human leishmaniases are expensive, have high systemic toxicity, and limited efficacy (Ghorbani & Farhoudi, 2018), more effective and user-friendly treatments are critical to cure this disease. In the Mediterranean region, *Leishmania infantum* is the specie mainly responsible for human leishmaniasis, being CL and VL its two main clinical manifestations.

We are developing innovative vesicular nanoformulations of low toxicity able to stably incorporate high amounts of antileishmanial drugs for their delivery to *Leishmania* parasites. Different nanovesicles have been designed carrying currently used drugs against *Leishmania* (amphotericin B, miltefosine, paromomycin, and pentamidine isethionate). Combinations of phospholipids were mixed with components specifically added to improve drug delivery. Those components were selected according to the administration route, which depends on the clinical form of the disease: for example, hyaluronic acid to improve topical administration in CL or sodium deoxycholate to increase the oral bioavailability of the drug in VL. Drug-loaded nanocomplexes have been tested *in vitro* against both *Leishmania infantum* developmental stages (promastigotes and amastigotes), and cell toxicity studies were performed. Some nanoformulations showed reduced cytotoxicity while keeping an antileishmanial effect similar to that of the non-encapsulated drug. The nanoformulations with best results will be tested in skin penetration and permeation experiments performed by means of Franz diffusion cells. Also, the *in vitro* lung deposition will be studied through the nebulization of formulations and measurement of aerodynamic behaviour.

On the other hand, we are developing DNA aptamers against *L. infantum* that will be used to increase targeting to parasite cells of drug-loaded nanovesicles.

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Improving the treatment of a dual-species *S. aureus* – *P. aeruginosa* mixed wound biofilm: impact of antibiotic and enzymatic therapy, and 3D spatial organization

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Chronic wounds are a relevant economic and health problem worldwide, which affects up to 2 million people in Europe. These wounds are associated with chronic polymicrobial infections, being *Pseudomonas aeruginosa* and *Staphylococcus aureus*, two of the most common opportunistic pathogens found in them. The microbial community in the wounds is embedded within a biofilm formed by the extracellular polymeric substance (EPS). The EPS is a network made of polysaccharides, proteins, lipids, and extracellular DNA (eDNA), which hinders the transport of antibiotics inside the biofilm and promotes the presence of microorganisms with antimicrobial tolerance. Due to this, it is necessary to investigate therapies that improve the penetrability and efficacy of antibiotics. In this context, our main objective is to study the interactions and colocalization of *P. aeruginosa* and *S. aureus* within the biofilm to understand their synergistic relationship. We used an optimized *in vitro* wound model that mimics an *in vivo* wound to coculture *P. aeruginosa* and *S. aureus*. Using this model, we demonstrate that antibiotic monotherapy differentially affects bacterial species in the mixed biofilm, driving one species to overcome the other, whereas dual antibiotic therapy efficiently reduces both species while maintaining a balanced population. In addition, we analyze the effect of different biofilm dispersion strategies, such as free and functionalized enzymes in nanoparticles, applied together with antimicrobial therapies. Antimicrobial tests show that DNase nanoparticle treatment has a potent antibiofilm effect, and □-amylase and cellulase have as well, but to a lesser degree. Finally, confocal microscopy images (CLSM) propose a three-dimensional colocalization model consisting of bacterial aggregates within the biofilm structure, which could be associated with the low efficacy of antibiofilm treatments on bacteria.

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Motion of Enzyme-powered Nanomotors in Complex Media

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The development of systems capable of precisely deliver drugs, which increase therapy efficacy and reduce side-effects has been a long-standing challenge in biomedicine. Biological barriers present in human body, such as the networks that compose the extracellular matrix (ECM), mucus or synovial fluid are one of the main challenges in the design of nanoparticles to treat tumors.[1,2] The highly complex structure of these fluids, composed of hyaluronic acid, glycoproteins and collagen can hinder the ability of conventional non-motile carriers to reach the target-site.[3] Thus, there is a clear need for disruptive new technologies and more efficient nanomedicines to increase efficiency.

Most of biological fluids are complex viscoelastic media, which means their viscosity is not constant and can suffer modifications. The flow behavior of such fluids can be altered when modifying their external conditions (eg. pH, temperature) or their internal structure (e.g. disruption of the network). In this sense, enzyme-powered nanomotors powered can potentially aid overcoming biological barriers using enzymatic catalysis of bioavailable fuels, given their continuous propulsion force and ability to modulate the surrounding environment. Bio-catalytic products released by this nanomotors and the substrates found in the surrounding media can lead to internal and external modifications.[4-6]

The resulting basic products arising from urease catalysis can induce pH changes[4], whereas for collagenase and hyaluronidase the catalytic reactions can lead to structural changes due to the network breakdown[7], improving both their ability to reach further locations than non-motile particles.

In this regard, we studied nanomotors powered by a different enzymes and their actuation within hyaluronic acid (HA), as well as biomimicked media fluids like simulated synovial fluid (SSF) and *in vivo* fluids, such real mucus extracted pig stomachs. Rotational test experiments using rheology revealed viscosity reduction in all complex viscoelastic structures tested. Oscillatory tests were carried out to study the complex media structure. The results showed not only that nanomotors actuation reduced stiffness, G' and G" values maintaining the stable structure.

Altogether, these results demonstrate that the bio-catalytic nanomotors can provide enhanced targeting capabilities to accomplish biological barriers. Their study and performance in biomimicking media and in complex environments will pave the way for the development of more efficient nanosystems.

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Enzymatic Nanomotors Towards Cancer Treatment

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Nowadays, most drugslack specificity and delivery efficiency, and show off-target effects. Nanomedicine's goal is to improve classical treatments, however nanosystems encounter difficult obstacles to overcome, such as biological barriers, that hinder greater efficacy. In this regard, nanomotors, which are particles capable of swimming in fluids, could potentially be an alternative solution to this unmet medical need.

Enzyme-powered nanomotors show improved diffusion and navigation within biological environments[1] and drug delivery efficacy,[2,3] compared to non-motile nanoparticles, and they can be used for a wide range of medical applications since they use endogenous fuels for their propulsion. In this sense, urease nanomotors have been chosen for treating urinary tract diseases as they are propelled with urea.[4,5] We have demonstrated that urease nanomotors modified with antibodies against FGFR3 exhibit improved targeting capabilities against 3D bladder cancer spheroids and that they reduce spheroids' proliferation compared to bare nanomotors.[5]

Nevertheless, large populations of nanomedicines are required to achieve high efficiency, and these nanosystems should be able to coordinate among themselves to perform their tasks optimally. Therefore, we investigated the collective behaviour of urease-powered nanomotors, both *in vitro* and *in vivo* using positron emission tomography. We observed that the collective motion of nanomotors not only leads to the generation of flows and enhances fluid mixing, but also allows the nanomotor populations to overcome hurdles in the form of turns and angles in confined paths. In addition, the intravesical administration of motile urease-powered nanomotors led to their homogeneous distribution within the bladder cavity, while passive nanoparticles were shown to remain at the instillation site.[6]

Apart from bladder cancer, other types of cancer have proven particularly challenging for traditional nanomedicine. For instance, nanosystems designed for treating lung cancer have shown a low efficacy in crossing the pulmonary mucosa. Catalase-powered nanomotors, which use hydrogen peroxide that can be found in the tumour as fuel, could potentially overcome the mucus biological barrier thanks to their active motion.

Altogether, our results prove that urease nanomotors improve targeting capabilities, as well as convection and mixing capabilities within living reservoirs. The research carried out also investigates the interaction between catalase nanomotors and a cell culture system that mimics the lung anatomy. We are confident that enzymatic nanomotors will help improve nanomedicine and overcome unmet clinical needs.

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Exploring Different Operating Regimes of Electrolyte-Gated Organic Field-Effect Transistor at the Nanoscale

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Abstract – Electrolyte-Gated Organic Field-Effect Transistor (EGOFET) is a fundamental building block of transistor-based biosensing platforms[1]. The nanoscale biorecognition events happening at their semiconductor/electrolyte or gate/electrolyte interface translates to significant changes in their global electrical characteristics. The study of these nanoscale-macroscale correlations demands a nanoscale tool to access the interfacial electrical properties. Recently, the In-Liquid Scanning Dielectric Microscopy technique has been shown as a promising tool to explore these interfaces in operating devices[2]. Further technical improvements coupled with automated data acquisition has resulted in a better understanding of their operating mechanism at the nanoscale. The present work explores an EGOFET as a function of drain and gate voltages as it goes through linear and saturation regimes. The onset of pinch-off and high gate voltage effects will be presented and discussed.

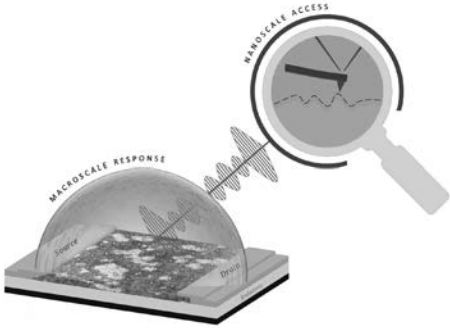

Figure: Illustrative representation of exploring EGOFETs with In-Liquid Scanning Dielectric Microscopy

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Utility of *Galleria mellonella* larvae for evaluating nanoparticle toxicology

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The use of nanoparticles in consumer products is currently on the rise, so it is important to have reliable methods to predict any associated toxicity effects. Traditional *in vitro* assays fail to mimic true physiological responses of living organisms against nanoparticles whereas murine *in vivo* models are costly and ethically controversial. For these reasons, this study aimed to evaluate the efficacy of *Galleria mellonella* as an alternative, non-rodent *in vivo* model for examining nanoparticle toxicity. Silver, selenium, and functionalized gold nanoparticles were synthesized, and their toxicity was assessed in *G. mellonella* larvae. The degree of acute toxicity effects caused by each type of NP was efficiently detected by an array of indicators within the larvae: LD_{50} calculation, hemocyte proliferation, NP distribution, behavioral changes, and histological alterations. G. mellonella larvae are proposed as a nanotoxicological model that can be used as a bridge between *in vitro* and *in vivo* murine assays in order to obtain better predictions of NP toxicity.

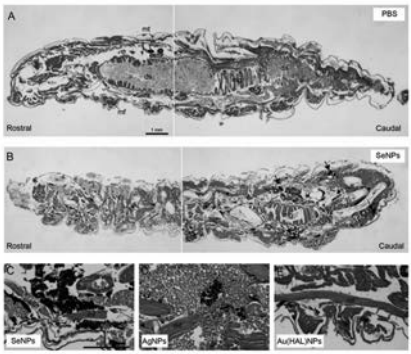

Figure. Histological characterization of the distribution of Se, Ag, and Au(HAL) nanoparticles injected in G. mellonella larvae.

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Upgrading the engine-chassis complex of micro-/ nanomotors towards application-oriented designs

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Enzyme-powered micro- and nanomotors self-propel by the biocatalytic conversion of substrates into products and show great promise as active biomedical[1] and environmental tools.[2] However, the limited library of engines and chassis studied needs to be expanded and the selected designs optimized for their specific implementation.[3] For this reason, we aim to explore certain engine and chassis enhancements with appealing properties that improve the self-propulsion of enzymatic micro- and nanomotors and make them more suitable for downstream applications, which include targeted drug deliver.

To improve the engine part of the micro-/nanomotors, we have improved the purity of the enzymes used to propel the particles. Commercial preparations of enzymes contain protein contaminants, thus, by purifying the enzyme, we have been able to increase the loading efficiency of our enzyme engine onto the microparticles, as well as quantifying the deleterious effect of protein impurities on the motion of the micromotors. Urease purification improves the speed of the micromotors 3- fold when compared to micromotors functionalized with unpurified urease.

As more specific application-oriented upgrades we also explore the appealing advantages that novel materials offer to build the micro-/nanomotor chassis. Metal-organic frameworks are used to obtain a multisized porosity that can be exploited to encapsulate the catalase (enzymatic engine) inside the mesopores while using the microporosity to capture a smaller contaminant molecule, hence, decontaminating while navigating.[4]

This work represents a compilation of micro-/nanomotors upgrades that first, improve the motion performance by increasing enzyme loading through the elimination of undesired proteins, and second by applying an engine-chassis design optimization to enhance their implementation in specific application. Altogether, these results provide new insights into the feasibility of implementation of enzyme-powered micro-/nanomotors and the understanding of its motion mechanism.

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Optimizing Enzyme Encapsulation in Targeted Nanoparticles for Enzyme Replacement Therapy of Lysosomal Disorders

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Lysosomal disorders arise from genetic deficiency of lysosomal enzymes, which leads to multi- organ diseases. Replacement therapy (ERT) by intravenous infusion of recombinant enzymes is a suitable treatment option; yet, is plagued by poor biopharmaceutical performance. In previous studies we have shown that coupling therapeutic enzymes to polymeric drug nanoparticles targeted to intercellular adhesion molecule 1 (ICAM-1), a receptor expressed on most disease cells, enhances their delivery in cell cultures and animal models. However, previous proof-of- concept formulations involved surface-coating of enzymes while encapsulation represents a more translational option. In this study, we use the double emulsion-solvent evaporation method to formulate biodegradable poly(lactide-co-glycolide) (PLGA) nanoparticles (NPs) for enzyme encapsulation of model hyaluronidase (HAse). First, we compared three copolymers, Resomer, Lactel I and Lactel II, and selected Lactell II because while rendering similar size, ζ-potential and encapsulation efficiency, these NPs showed lower polydispersity index (PDI). Then, using this copolymer we compared surfactants PVA, DMAB and Pluronic F68, and selected F68 because it rendered significantly negative ζ-potential, a feature shown to prevent aggregation and interaction with some serum proteins. Next, we modified the enzyme input for NP preparation, the presence of carrier proteins such as albumin, and the enzyme-to-carrier protein ratio. All resulting formulations had relatively similar size, PDI and ?-potential, while enzyme encapsulation broadly varied. This study allowed us to select the formulation which best adjusted to all required parameters, which had 180 nm diameter, 0.1 PDI, -36 mV ?-potential and 92% encapsulation efficiency, which contained 538 enzyme molecules per NP. These NPs showed enzymatic activity at intended lysosomal conditions, could be coated with antibodies targeting ICAM-1 (121 molecules/NP after serum incubation), were lyophilized for storage and reconstituted without affecting their main properties, and were relatively stable under storage conditions. Furthermore, these NPs interacted with ICAM-1-expressing cells and markedly enhanced enzyme delivery compared to both enzyme-coated NPs formulations and free enzyme control. Therefore, these NPs represent attractive formulations for lysosomal enzyme replacement therapies.

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Spanish Ministry of Science and Innovation project RTI2018–101034-B-I00 and National Institutes of Health project R01-HL98416.

Mapping the Nanoscale Dielectric Properties of Cells by Scanning Dielectric Force Volume Microscopy and Machine Learning

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Mapping the dielectric properties at the nanoscale of samples showing a complex topography, such as single cells, poses formidable challenges to existing nanoscale dielectric microscopy techniques [1][2]. Here, we will explain how these limitations can be overcome by introducing Scanning Dielectric Force Volume Microscopy (SDFVM) and machine learning [3,4]. SDFVM is a scanning probe microscopy technique based on the acquisition of electrostatic force approach curves at every point of a sample and its post-processing and quantification by using a computational model that incorporates the actual measured sample topography. The technique provides quantitative nanoscale images of the local dielectric constant of the sample with unparalleled accuracy, spatial resolution and statistical significance, irrespectively of the complexity of its topography. The potential of the technique is illustrated by presenting the first nanoscale dielectric constant map of a single bacterial cell [3] and of eukaryotic cells [4], including its small-scale appendages and nanoelongations. The high quantitative accuracy and spatial resolution of the map enables identifying different dielectric properties of the cell wall and of the cytoplasmatic region, as well as, the existence of variations in the dielectric constant along the bacterial cell wall itself. We then show that the computation times required to extract the nanoscale dielectric constant maps, which with conventional means can be of the order of several weeks, can be decreased dramatically down to seconds, by using supervised neural networks. This approach opens the door for nanoscale composition mapping of eukaryotic cells and other complex biological samples with scanning dielectric microscopy. Scanning Dielectric Force Volume Microscopy is expected to have an important impact in Materials and Life Sciences where the mapping of the dielectric properties of samples showing complex nanoscale topographies is often needed.

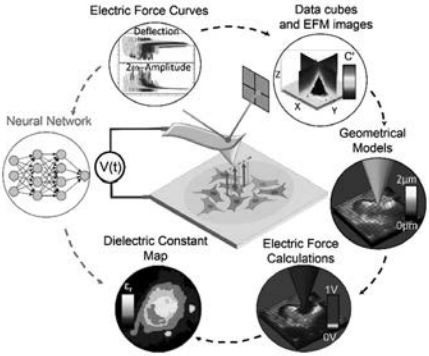

Figure 1: Schematic representation of Scanning Dielectric Force Volume Microscopy applied to the dielectric constant mapping of eukaryotic cells [4].

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Cell mechanics influences the mammalian circadian clock via YAP/TAZ

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Most organisms on Earth are subjected to a 24-hour oscillatory behaviour due to their adaptation to the daily pattern of sunlight. In mammals, those oscillations emerge from a molecular clock contained in almost every cell of their body. The different cellular clocks are subjected primarily to the control of the light-sensitive suprachiasmatic nucleus, but they work and are regulated independent and locally. Indeed, recent studies have estimated that around the 10% of the proteins have a time-dependent expression that is cell type specific.

We aim to study the local regulation of the fibroblast circadian clock, specifically addressing the influence of mechanobiological hallmarks. We have used NIH3T3 stable cell lines expressing Venus fluorescent protein under the promoter of the core circadian protein RevErb, confocal microscopy and customised computational analysis. Our results indicate that RevErb basal levels and circadian oscillations in NIH3T3 cells depend on cell density. By carrying out wound healing experiments, we have seen that RevErb expression, typically low and rhythmic, is perturbed as soon as cells start migrating.

To disentangle the pathway that triggers those changes in RevErb expression upon cell density changes, we used PRIMO, a novel technique to micropattern extracellular matrix proteins on hard substrates, to mimic a confined environment for single isolated cells. We noted that confined isolated cells displayed RevErb circadian oscillations similar to those of overconfluent cells. Next, we stopped the migration of single cells by altering their actin cytoskeleton dynamics with jasplakinolide and latrunculinA and observed the striking emergence of robust circadian oscillations, unlike the case of untreated single cells.

We then checked the localization of the two most important mechanosensitive transcriptional regulators, YAP/TAZ and MRTFA, in the aforementioned diversity of conditions. Surprisingly, we observed that the circadian strength of all the studied cell populations anticorrelates with their nuclear levels of YAP/TAZ. However, there is no relation with those of MRTFA. We subsequently tested if YAP/TAZ has a direct role in the regulation of the mammalian circadian clock overexpressing dominant positive mutants of YAP and TAZ which are functional but localised constant and predominantly at the nucleus. Those cells displayed a dramatic impairment of the RevErb clock, demonstrating a novel role of YAP/YAZ intracellular localization as a circadian modulator.

Biomechanics of the progression of hypermethylated colorectal carcinomas

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According to the World Health Organization, cancer is one of the main causes of death worldwide, with colorectal carcinoma (CRC) being the second-leading cause of tumour related-death. The high rate of mortality of CRCs is principally attributed to the metastasis of neoplastic cells from the primary tumour to secondary organs such as the liver, the lung and the peritoneum. The molecular and cellular mechanisms underlying CRC metastatic invasion of the peritoneum have been described extensively by Zajac et al. This study revealed a novel cellular modality of peritoneal metastatic spread characterized by the presence of large clusters of cancer epithelial cells displaying an outward apical polarity. These clusters of cells, termed tumour spheres with inverted polarity (TSIPs), originate through a series of morphological changes. The first event is the sprouting of hypermethylated epithelia, followed by their apical budding, leading to the formation of rounded spherical clusters of cells called buds, and the subsequent cleavage of the newly formed spheres. How cell and tissue mechanics drive this process is still unclear. To provide novel insights into this metastatic cascade, our project aims at deciphering the biomechanical events regulating the formation of buds in colorectal cancer cell lines. Employing a combination between cellular biology techniques with biophysical methods, we showed that this process is caused by increased contractility of epithelia, coupled with the formation of topological defects at the level of the monolayer underneath the buds.

Our study demonstrates that buds development in colorectal carcinomas epithelia is governed by morphological transitions occurring entirely at multicellular level, rather than by single cells aggregation.

Studying mechanotransduction in the nucleus: a multicellular approach

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Environmental and cellular forces affect cell behaviour, homeostasis, and development. Mechanotransduction happens when cells transduce these forces intoto biochemical signals, which in turn regulate transcription. To understand how this happens, we have designed and tested a sensor of forces applied to the nucleus, based on our finding that active transport between the cytoplasm and the nucleus depends on force. The sensor is a fluorescent protein undergoing active transport into the nucleus, which changes its nuclear concentration when force is applied to the nucleus. The readout of the sensor is the nuclear to cytoplasmic ratio of the fluorescence emitted by the bulk of proteins. Upon forces getting to the nucleus the sensor translocates to the nucleus and the nuclear to cytoplasmic ratio increases. The sensor was tested in single cell Mouse Embrionary Fibroblast conditions and now we are carrying a multicellular approach with MCF10A monolayers to understand how cells in monolayers are affected by forces in the nucleus. For this, we compute sensor ratio, cell density, nuclear shape, nuclear size, and also inhibit specific force generators in the cell with precise drug treatments.

Fibrillar adhesions confer mechanical memory to the nucleus

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Forces applied to cell nuclei are increasingly recognized as a major driver of cell function. As forces reach the nucleus, cells thus require buffering mechanisms to sustain them or dissipate them in a controlled way, but how this occurs is largely unknown. Here we show that the remodelling of fibronectin and the simultaneous formation of fibrillar adhesions, gives rise to a network of actin stress fibres under the nucleus. This fibrillar network locks the nucleus into a mechanically active conformation that is resistant to sudden changes in the mechanical environment of the cell, encompassing both a rapid increase or decrease in the mechanical stimuli. Our results reveal a new mode of mechanical memory whereby the nucleus is held in place by a long-lived network of fibres.

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. These force patterns co-evolve with fate specification to progressively shape a mature compartmentalized epithelium. The size of the stem cell compartment depends on ECM stiffness and on endogenous cellular forces. 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 compartmentalization, folding and collective migration in the intestinal crypt.

Cancer stem cell and the metastatic cascade: a mechanical approach

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The organization of colorectal cancer (CRC) is reminiscent of normal intestinal tissue, with CRC tumors often composed by heterogeneous populations comprising more differentiated cells and a small pool of cancer stem cells. The role of intestinal cancer stem cells in tumor growth and treatment resistance has been the focus of extensive investigation, with many studies pointing to microenvironmentally defined plasticity and interchangeable cancer differentiation states. Nevertheless, the role of cancer stem cells in the metastatic process remains still to be fully elucidated.

Patient-derived Organoids (PDOs) are 3D structures, derived from polyclonal biopsies, which self-organize and can resemble, up to a certain extent, the *in vivo* architecture and cellular heterogeneity. Using PDOs engineered to fluorescently label cells expressing LGR5, a well-established marker for CSCs, we performed a broad biophysical characterization of the PDOs cancer cell populations based on their stemness level. To do so, we adopted a bottom-up approach ranging from a single-cell level to higher complexity levels such as cluster level and interactions with endothelial cells.

At the single cell level, we observed differences in cell shape and rotational asymmetry of the applied stress field representing the cellular polarization state. LGR5+ cells displayed a more elongated and polarized shape while the LGR5- cells exhibited higher circularity and a smaller asymmetry in the stress field. Those differences translated in different migratory and morphological phenotypes at a cluster level, as assessed by observing the cluster collective invasion features on gels. Clusters expressing high levels of LGR5 showed a more spread and flattened shape characterized by lower roundness compared to more differentiated clusters. Moreover, LGR5 expression in the clusters was negatively correlated with their migration speed and their polarization state. Hence, clusters containing more differentiated cells were found to migrate faster, display higher roundness and higher polarization state.

To further proceed with the CRC biophysical characterization, we investigated whether LGR5 expression in CRC clusters affects their ability to adhere to an endothelial monolayer and form a gap through which they can attach to the underlying collagen coating. Notably, we found that clusters expressing more LGR5 have an advantage while attaching to the endothelium as indicated by higher attachment rate and shorter time to form a gap.

In the current study we show that indeed CRC cells differentiation states cells are correlated to distinct biomechanical phenotypes that may impact their metastatic potential. We intend to further investigate the mechanism behind these phenotypes through different molecular approaches.

Capacitive electrical stimulation promotes neuritogenic signaling

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Keywords: Central nervous system injuries; electrical stimulation; capacitive coupling; neuronal differentiation; neuritogenesis; cellular signaling.

Due to the poor regenerative ability of the central nervous system when injured, and the lack of effective regenerative treatments, innovative therapies are thus a priority. Electrical stimulation has been the focus of attention as a possible therapeutical intervention, capable of improving neuronal differentiation and regeneration. The work here presented, aimed to test the effects of delivering electrical stimulation when using our innovative cosurface capacitive system (CCS) on the course of normal neuronal differentiation. An electrical stimulation of 20 Hz, 200 mV/mm and 100 µs pulses for 1 hour/day[(1)] was delivered by our CCS[(2–4)] to SH-SY5Y cells, undergoing neuronal differentiation with retinoic acid and BDNF. Phase contrast microphotographs of control and stimulated cells were measured to study neurite outgrowth, and the levels of proteins known to be involved in neuritogenic signaling pathways (GαO, pSTAT3/STAT3 and pERK/ERK) were analyzed by immunoblot. Morphometric analyses revealed that neurite outgrowth was increased in cells under stimulation. In agreement, GαO levels and both the pSTAT3/STAT3 and pERK/ERK ratios were increased by the capacitive stimulation. While the activation of the GαO-STAT3 pathway occurred earlier during the differentiation process, pERK1/2 activation increased later. Summarizing, although this was the first capacitive stimulation assay on neuronal-like cells performed with this apparatus, results obtained so far already suggest that the system has neuroregenerative potential. More experiments will be performed to increase the sample size, confirm significant alterations in neuritogenesis, to further optimize the stimulation settings before testing the system in neuronal regeneration assays.

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Cells exert pushing forces on their environment

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The importance of mechanicals signals in processes like embryogenesis, wound healing or cancer is now clear, and has given rise to the field of mechanobiology. Studies of cells seeded on two- dimensional (2D) flat surfaces have allowed researchers to assess cell force transmission patterns, and the cell components required for both force transmission and transduction into biochemical pathways. However interesting, these systems are limited by the fact that they do not recapitulate three-dimensional (3D) cell shapes, which play a major role in cell responses.

In this work, we present a new system made of polyacrylamide wells that enables the measurement of single-cell force transmission and transduction in 3D. With it, we report a novel phenomenon in which cells exert pushing forces on their environment, and not only pulling forces as previously described. This behaviour is correlated with their volume. This phenomenon raises the question of how these forces are generated and propagated through the cell, as well as how it impacts mechanotransduction events in 3D physiological environments.

Inhibition of the immunosuppressive effects of activated fibroblasts in non-small cell lung cancer

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Lung cancer is the leading cause of cancer death, with an 18% survival at 5 years of diagnosis. However, it is expected that this survival will improve thanks to recent advances in immunotherapy, which have shown long-term therapeutic responses in selected patients in advanced stages for the first time. Currently, immunotherapy aims at patients with high expression of the immune checkpoint PD-L1, but for unknown reasons, there are patients who show poor response or acquire resistance to anti-PD-L1 treatment, suggesting the existence of alternative immunosuppressive mechanisms to PD-L1. Recent studies have shown that activated/myofibroblast-like tumor-associated fibroblasts (TAFs) may play a key immunosuppressive role, including the secretion of immunosuppressive factors that reduce the activity and/or recruitment of CD8+ cytotoxic T cells and favor the recruitment of immunosuppressive cells (macrophages-M2 and myeloid-derived suppressor cells (MDSC)), as well as the expression of PD-L1. In addition, the deposition of fibrillar collagens by activated TAFs leads to a marked stiffening of the tumor, favoring hypoxia, which can by itself increase the activity and/or the recruitment of T-regulatory lymphocytes and stimulate the overexpression of PD-L1 in cancer cells. Here we began to analyze the immunosuppressive effects of lung TAFs in culture, and found that TGF-b1- activated TAFs can promote an immunosuppressive microenvironment both by increasing the expression of immunosuppressive factors like IL-6 and by promoting the expression of PD-L1 on cancer cells.

Development of a model of "macro" substrates for the analysis of 3D chromatin structure and transcriptional profiling

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Mechanically-induced changes in the genome are increasingly recognized as major drivers of cell and tissue function. However, current and past studies of this topic *in vitro* have often been limited by the sample size required for these genomic analyses. Here we describe the development of a polyacrylamide gel (pAAg) substrate with larger area than gels previously generated in labs world- wide. These substrates display the same tunable stiffness as their smaller counterparts and are particularly suitable to accommodate large numbers of cells. We tested the substrates to assess the effect that changes in rigidity have in the cells' genome, both at the level of chromatin organization and transcriptional regulation. For this, two different rigidities were used (soft vs stiff) with three conditions which included: i) a control group, ii) a transiently expressing mutant of RanQ69L that prevents all nucleocytoplasmic transport and iii) a transiently expressing mutant of NES1-KASH that prevents force transmission to the nucleus. Our preliminary results suggests that cells seeded on pAAg substrates of different rigidities display differences in chromatin structure and gene expression, but more data will be necessary to further support these results. Ultimately, the aim of our project is to understand down the road how changes in rigidity affect: i) the 3D structure and interaction map of chromatin and ii) the activated or repressed transcriptional programs in soft and stiff substrates. Overall, this new model will help us to characterize how the genome is affected by rigidity both at the structural and transcriptional levels. More broadly, we expect the potential findings of this work to help the community detailing the effect of changes in tissue stiffness in the genome spatial organization.

A novel durotactic migration emerges from the proximity to a wetting transition

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Directed cell migration –the ability of cells to follow environmental cues– is an essential mechanism 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. Recent studies suggest that E-cadherin, a cell-cell adhesion protein that is essential to maintain tissue integrity, promote coordination and establish cell polarity, could govern cell migration in ECM-depleted environments. However, how cells are able to orient themselves to undergo directed migratory events in the context of cadherin-mediated migration still remains unknown. In this work, we show that the migration of human epidermoid carcinoma cell clusters (A431) on hydrogels coated with oriented E-cadherin molecules is characterized by drastic transitions in tissue morphology. Moreover, we report for the first time that E-cadherin-mediated migration triggers strong durotactic migratory events, a form of directed cell migration in which cells follow stiffness gradients. We show that E-cadherin-mediated durotaxis increases with cluster size and shows a biphasic relationship with stiffness offset. Interestingly, we observed that some A431 cell clusters migrate unexpectedly large distances, presenting a non-gaussian migratory behavior, a hallmark of ultrafast dynamics. In analogy with the behavior of inert fluids, we have interpreted the drastic morphological transitions experienced by migrating A431 clusters as active wetting transitions, arising from the interplay between traction forces and contractile intercellular stresses. Intriguingly, durotaxis was maximal in clusters whose wetting state was in the proximity of a wetting transition, enhancing their ability to follow mechanical cues. Following our observations, we took a coarse-grained approach to develop a model based in active matter aiming to explain how collective cell migration depends on the physical forces as a function of tissue size, cell contractility, cell-substrate adhesion, substrate stiffness offset and stiffness gradient slope. Interestingly, the model predicted that A431 cell clusters migrating on fibronectin substrates should display a similar phenomenology if contractility was promoted. We found

that after challenging clusters with human Epidermal Growth Factor (hEGF), a growth factor that increases cell contractility, the migration on fibronectin-coated hydrogels reproduced the features observed in E-cadherin-coated substrates. To date, durotaxis has only been described on ECM-coated surfaces, such as fibronectin and collagen. In this work, we not only show that E-cadherin-dependent migration results in active wetting transitions that enable durotactic behaviors, but also that this migratory mode emerges from a fine balance between adhesion and contraction and can be generalized to all kind of substrate coatings by altering the wetting state of clusters.

A mechanobiological study of pluripotency dissolution in mouse embryonic stem cells

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Pluripotency, the ability to generate all organs and tissues, exists as a spectrum of states in mouse embryo. When it is newly established, as is the case in the inner cell mass (ICM), pluripotency is homogeneous and is referred to as naïve state. Importantly, in this state, cells are devoid of differentiation competence and to acquire functional maturity must shed their naïve identity. Monitoring this process in the embryonic context is technologically challenging and is therefore studied *in vitro* through Mouse Embryonic Stem Cells (mESCs). They replicate and perpetuate ICM's naive pluripotency when cultured in defined N2B27 media with two inhibitors (2i) for Mek/Erk and GSK3β signaling. For naïve state exit, the instructive signal is the integrin mediated mechano-sensing of extra cellular matrix (ECM), while 2i withdrawal is only a permissive cue. Among the various ECM ligands, Laminin was identified to be the pivotal one for directing naïve identity loss. However, the mechano-responses accompanying laminin sensing, their spatio-temporal evolution and regulatory role in pluripotency maturation have remained unknown. Here in this work, we cultured mESCs on laminin functionalized polyacrylamide gels. The employed mESC line *Rex1::GFP* possesses a fluorescent reporter whose signal gradually decreases upon naïve state exit. When surveyed for various mechano-responses after 2i withdrawal, we observed a progressive increase in traction forces and lengths of focal adhesions. Additionally, as pluripotency dissolved, the basal actin reorganized from mesh like network into oriented filaments resembling stress fibers. Furthermore, treatment with blebbistatin, the non-muscle myosin-II inhibitor, significantly delayed the fluorescent signal decay, hinting at the potential regulatory role of cell contractility in driving mESC maturation. Finally, the nuclei of naïve exited cells displayed flat morphology, a sign of mechanical coupling with cytoskeleton. In other stem cell systems, nucleo-cytoskeletal coupling is shown to be mediated by KASH domain proteins. Hence, by targeting them, the current focus is to intervene in mechanical signal relay and elucidate its role in pluripotency dissolution.

Lung and Cardiac Microvascular Tissues on-Chip Reveals Differential Fibrotic Phenotypes

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Tissue fibrosis— which is classically indicated by the excess deposition of extracellular matrix proteins and increased tissue stiffness—is implicated in various cardiac and pulmonary diseases. Its pathophysiology is thought to stem majorly from local fibroblast activation in the context of stroma-parenchyma interactions; however, recent findings have implicated stroma-endothelial crosstalk in pathological fibrosis. To our knowledge, the use of functional vasculature to replicate cardiac and lung-specific fibrotic hallmarks has not been reported. We developed an on-chip vascularized 3D model to investigate stroma-endothelial crosstalk in normal and fibrotic conditions within cardiac and pulmonary-like environments. By employing human-induced pluripotent stem cell-derived endothelial cells (hiPSC-ECs) co-cultured with (and without) primary human cardiac and lung fibroblasts in a microfluidic device, perfusable cardiac- and pulmonary-like microvascular tissues were generated. Vascular endothelial barrier function, vascular morphology, and extravascular matrix properties (measured by diffusivity and nanoindentation) were differentially impacted by the stromal cell type. These microvascular tissues appear to take on a wound-healing state, indicated by expressed inflammatory cytokines— and vary in their inflammatory profile. Subsequent administration of transforming growth factor- β (TGF-β) induced varied fibrotic phenotypes, with the lung co-culture resulting in increased stiffness, increased alpha-smooth muscle actin expression, and reduced MMP activity. Our findings highlight the significant impact of stroma-endothelial interactions on microvessel development and extravascular matrix remodeling in normal and fibrotic states. This humanized 3D *in vitro* model presents an opportunity to study distinct differences in cardiac and pulmonary fibrosis and could be used employed in the development of patient-specific anti-fibrotic therapies.

Osteogenic differentiated adipose-derived stem cells create an *in vitro* bone model inside microfluidic platforms

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In vitro models of bone tissue have proved themselves to be a potential tool to understand bone mechanobiology processes, offering a wider comprehension of biological mechanisms[1].

Stem cell based therapies for tissue repair are becoming more important in regenerative medicine. Mesenchymal stem cells are widely used in stem cell based therapies due to their multipotent capacity, able to differentiate into different tissues, bone between them. Among other options, human adult adipose tissue may represent a suitable stem cell source as it has been demonstrated before[2].

In this study, a microengineered bone model has been created out of Adipose-Derived Stem Cells (ADSC). ADSC were differentiated in osteogenic culture medium and, afterwards, seeded inside microfluidic platforms embedded in rat tail type I collagen hydrogel at the final concentration of 10^6 cells/ml. Then, they are cultured up to 21 days.

Flow cytometry, DNA content, extracellular alkaline phosphatase (ALP) activity assay, dendrite tracking, calcein staining, BSP2 and DMP1 protein immunofluorescent staining were conducted to study cell type and morphology evolution[3].

ALP activity and specific protein release show the stem cells osteogenic transition and proves that mature bone cells are obtained at the end of the culture. Thus, full osteogenic differentiation has been successfully achieved inside the microfluidic device. Collagen hydrogel embedding bone cell niches proves that are able to replicate bone tissue environment *in vitro*. This fact offers a powerful tool to study, inside a thoroughly controlled atmosphere, bone cells responses against different stimuli.

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Dynamics of stem cell migration and condensation on nanopatterned adhesive ligands

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Cell migration is an essential part of biological processes such as development, wound healing and disease progression. It is the main regulator of mesenchymal condensation, a prevalent morphogenetic transition in which mesenchymal stem cells (MSCs) gather to form three-dimensional structures that set the architectural foundations of tissues such as cartilage. Here we analysed single and collective cell migration in a model of early chondrogenic development, starting with single undifferentiated MSCs and progressing towards multicellular aggregates, or condensates.

Cell-substrate interactions are driven largely by integrin receptors at the plasma membrane; integrin- mediated adherence with extracellular RGD ligands is a paramount factor modulating cell migration. We have previously shown that RGD-nanopatterned substrates can be tuned to modulate MSC adhesion, differentiation, mechanotransduction and intercellular communication during cartilage formation [(1-4)]; here we use RGD nanopatterns to study the influence of local ligand density on MSC migration dynamics. We live imaged the first 40 hours of condensation to quantify cell displacement, speed and directionality, as well as the rate of cell-cell collisions; and we pharmacologically blocked cell-substrate and cell-cell interactions to investigate their role in the regulation of single and collective cell migration.

Nanopatterns of intermediate ligand density lead to faster movement and more cell-cell collisions, facilitating the condensation process, whereas a full protein coating impedes it by making cells slower and highly directional. Migration of whole cell condensates also depends on ligand density. Blocking certain sets of interactions differently affects single cell and condensate migration.

These findings provide insight on the regulation of single and collective stem cell behavior through nanoscale environmental cues during tissue development. They contribute to the design of nanobiomaterials for the regeneration of tissues such as cartilage, among other applications that require precise control over cell movement.

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Photo-crosslinkable hydrogels for skin model reconstruction

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Photo-crosslinkable hydrogels are the newest frontier in the field of biofabrication and represent one of the most suitable biomaterials for tissue engineering applications. Norbornene based thiol-ene systems are becoming incredibly attractive in light-based-3D-bioprinting due to the extremely fast thiol-norbornene photo-crosslinking reactions, resulting in a high degree of control on the number of reacted functionalities just varying the thiol-ene ratio.[1] In this study, a novel photopolymerization process based on visible-light 3D bioprinting is proposed with the aim of developing 3D *in vitro* skin tissue models, using norbornene based hydrogels. For this purpose, a Direct Laser Writing system (DLW), which consists of a 405 nm wavelength laser diode coupled to automated translational stage, has been employed, allowing the fabrication of skin-like scaffolds using reduced sample volumes. Among different polymer combinations analyzed, photo-crosslinkable Norbornene-pullulan hydrogels have been used, since pullulan shows excellent properties, mechanical stability, high hydration capacity and biocompatibility, which have been proved suitable for cell-based dermal replacement.[2,3,4]

To recreate the different skin layers, first, cell-laden hydrogels were fabricated mixing human skin fibroblasts (Hs-27) suspension with the pre-polymer solution. After photopolymerization, samples were assembled in Transwell® inserts and kept in standard culture conditions. A few hours after encapsulation, fibroblasts were fully elongated within the hydrogel matrix, with a cell viability higher than 70 % after one week, proving the high compatibility between cells and hydrogel composition.

Subsequently, human keratinocytes (HaCaT) were seeded on top of hydrogels, already containing Hs-27 embedded, to mimic the epidermal layer and kept in standard cell culture conditions up to 21 days. Air-Liquid Interface (ALI) culture conditions were applied to boost their growth, resulting in multilayered development. Cell adhesion onto hydrogels surface was promoted by the incorporation of RGD peptides into the pre-polymer solution. Immunostaining studies showed a uniform distribution of fibroblasts along the full thickness of the hydrogels, promoting interactions with HaCaT cells (Figure 1). A higher production of extracellular matrix (ECM) proteins was also revealed on the co-culture systems (Hs-27/HaCaT) compared to single fibroblast cultures, evidencing their crosstalk. These promising results do not only demonstrate the good compatibility between cells and the hydrogel structure but are also tracing an excellent pattern to reach a final skin model.

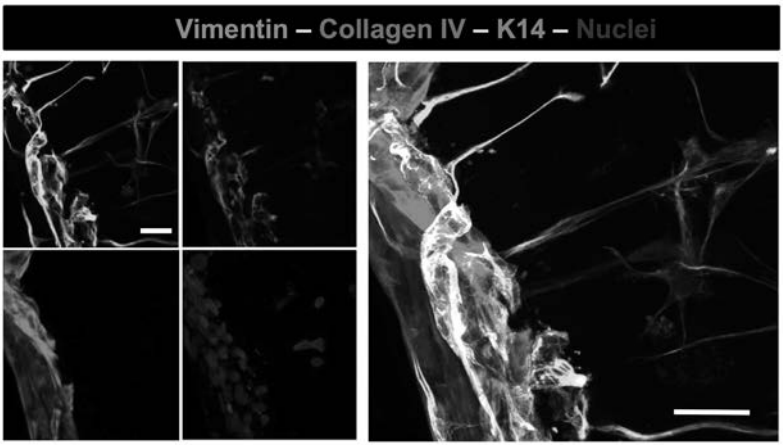

Figure 1. Confocal maximum intensity projections of immunostaining for Vimentin (green), Collagen IV (red) and K14 (magenta) on the co-culture of Hs-27 and HaCaT cells on hydrogel cross section (individual channels -left- and merged -right) 14 days after HaCaT seeding.
Scale bar = 25 μm

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Flexible Magnetic 3D Printed Robots for Cell and Drug Delivery

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The actuation of microrobots by using external magnetic fields allows the untethered control of such microdevices and have the potential to make medical operations less invasive and more precise. They are promising for many applications, such as targeted therapy or minimally invasive surgeries. Current challenges in the field of magnetic microrobotics include efficient propulsion, biocompatibility and highly precise functionality for advanced manipulation purposes.

Our work addresses at first the optimization of the flexibility of a soft magnetic swimmers fabricated from PDMS by tuning its material properties. Second, the influence of the location of its magnetic segments on the forward propulsion by magnetic actuation is studied (Fig.1A). Also, its biocompatibility and potential as cell delivery machines is investigated (Fig.1B).

Further, we explore other materials with advanced properties such as stimuli-response to temperature, allowing on-demand adaptability of their shape and triggered cargo release. Poly-N- isopropylacrylamide is presented as one optional material to obtain shape-changing structures by 3D printing this stimuli-responsive hydrogel and applying reversible temperature triggers (Fig.1C). These adaptive microrobots can be explored for cargo loading and release purposes. The addition of gold nanorods allow the laser-triggered thermoresponse by photothermal mechanism and thereby release of cargo (Fig.1D).

These preliminary investigations aim to pave the way to develop flexible magnetic and highly functional magnetic robots for cell and tissue delivery and on-demand cargo delivery within the frame- work of the La Caixa Junior Leader program. This project welcomes interactions with experts from microfabrication of biomaterials and regenerative medicine.

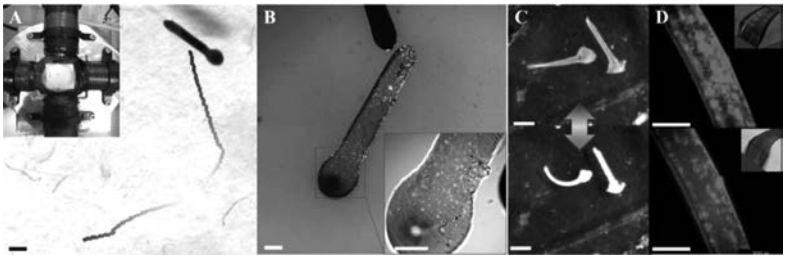

Figure 1: Flexible magnetic robots for cell and cargo delivery. A) Swimming path of elastomeric PDMS filament actuated by oscillating magnetic fields generated by electromagnetic coil setup (inset). Scale bar 2mm. B) Skeletal muscle cells grown on PDMS showing its biocompatibility and cell cargo ability. Scale bar 500µm. C) Shape change of 3D printed PNIPAM structures when changing temperature from 30°C (top) to 38 °C (bottom). Scale bar: 2mm D) Release of fluorescently labelled microparticles (red) from PNIPAM filaments when 747nm light is applied for 8 minutes. Top image: Before light exposure, bottom: after light exposure. Inset shows cross-section of z-stack. Scale bars 200µm.

Effect of lactate on cardiac fibroblast activation and its implications for in situ cardiac regeneration

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Cardiac fibroblasts (CFBs) have a key role during cardiac remodeling after myocardial infarction due to their ability to secrete and breakdown the extracellular matrix. In response to cardiac injury, CFBs become activated, proliferate, migrate, provide mechanical support, and contribute to a myriad of physiological signaling processes. Initially, all these processes are essential to the reparative wound healing response; however, over time these effects become maladaptive leading to fibrosis and cardiac dysfunction. Given the phenotypic plasticity and functional diversity of CFBs, we aim at modulating CFBs activation to restrain fibrosis after injury. Specifically, we focus in unraveling the effect of lactate on CFB activation. 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, being proposed as a novel bioactive signal for *in situ* cardiac regeneration therapies [1].

Primary adult human CFBs were exposed to exogenous lactate at different concentrations. CFBs metabolic activity and toxicity after exposure to lactate were assessed. The inflammatory and fibrotic responses to exogenous lactate were investigated in terms of cytokine and collagen production, cell proliferation, cell migration assays, and myofibroblast differentiation. Furthermore, CFBs were treated with TGF-β1 to induce myofibroblast transition for proper comparison with the cultures unexposed and exposed to lactate.

Our results indicate that exogenous lactate significantly reduces the expression of detrimental cytokines for cardiac repair, the expression of the main myofibroblasts markers, and it delays the characteristic migratory profile of activated fibroblasts. Furthermore, lactate-treated CFBs show no significant differences in terms of proliferation, collagen production, or contractility properties when compared to untreated control CFB cultures. This suggests that the potential benefits observed in lactate-treated samples are compatible with the primary regenerative function of the CFBs.

Lactate has been recently proposed as a novel bioactive signal for endogenous cardiac regeneration therapies. In this study, we have characterized the effect of lactate on CFBs, the main cardiac cell type involved in ventricular remodeling after injury. Exogenous lactate seems to prevent myofibroblast differentiation without compromising their activation and viability, and thus could hamper sustained fibrosis in prospective clinical applications. Altogether, this study further supports the potential use of lactate for *in situ* cardiac regeneration treatments.

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Design of experiments to fabricate hydrogels by visible light photopolymerization using a 3D bioprinter

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#Equal contribution

Hydrogels have been investigated as a simple strategy to engineer 3D models because they provide friendly environments for cell growth and differentiation with a broad range of mechanical properties[1]. Among the different techniques to manufacture these biomaterials, photopolymerization in conjunction with 3D bioprinting offers unique advantages such as spatial addressing and control of the light source that allow to produce stable and smooth hydrogel networks in a short time. Regarding the photopolymerization process, it is important to select a suitable photoinitiator since the polymerization of the hydrogel and its functionality will depend on this. Compared to UV sensitive photoinitiators, visible light-sensitive photoinitiators are less cytotoxic and are highly soluble in water[2]. However, polymerization is a complex process, and to find the proper combination of prepolymer solution composition and printing parameters can be time and money consuming, thus the use of Design of Experiments (DOE) is useful on optimization process and find variables that interfere more in the answers[3]. With this in context, this study aims to use experimental design to try to find the best combination to fabricate photo-crosslinkable gelatine methacryloil (GelMA)— poly(ethylene glycol) diacrylate (PEGDA) hydrogels in the presence of a visible light source and the photoinitiator eosin Y, the co-initiator triethanolamine (TEA) and co- monomer N-vinylpyrrolidone (NVP). To define the best concentrations and printing parameters for the hydrogel, two factorial designs 23 and 24 were carried out. In both cases, the normal layer exposure time was the most significant factor (~78%) (Figure 1) and good-shaped hydrogels were obtained when its value is shorter. Related to the prepolymer solution composition, better results were obtained when TEA concentration was lower. To evaluate the mechanical properties of the hydrogels fabricated with the best prepolymer solution found in the DOE, swelling assay and Young's Modulus measurements were carried out, obtaining a maximum swelling value of 6.63% (reached after 1h) and Young's Modulus values close to 0.6 ± 0.02 kPa. Moreover, cell viability and immunofluorescence assays showed that NIH-3T3 fibroblast cells grew and proliferated throughout the hydrogel without affecting its function (Figure 2). Here, we demonstrated that the use of DOE was adequate to set good parameters to fabricated GelMA-PEGDA hydrogel networks with mechanical and biochemical characteristics necessary for the growth, proliferation and development of NIH-3T3 fibroblasts for long-term 3D cultures.

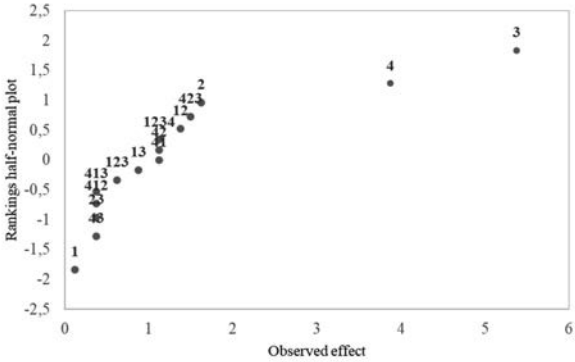

Figure 1. Half normal plot for the Design of experiment 24, effects: 1 (prepolymer solution), 2 (Layer Thickness), 3 (Bottom layer exposure time) and 4 (Normal layer exposure time).

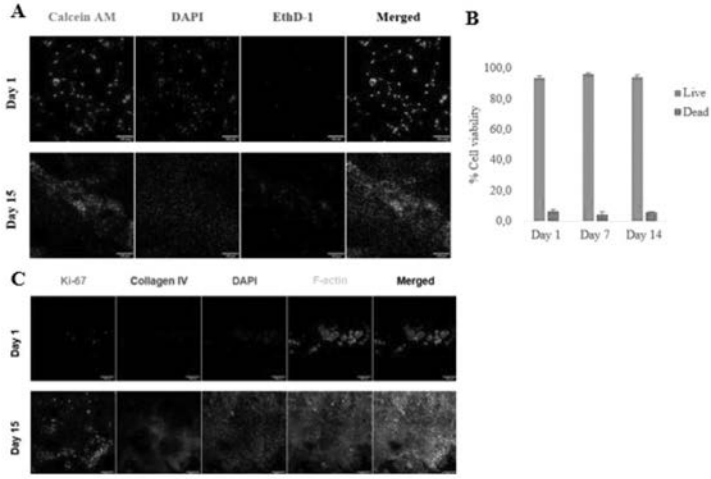

Figure 2. Live / Dead assay for NIH-3T3 cells embedded into hydrogels after 1, 7 and 15 days. (A) Confocal images stained with Calcein AM (green), DAPI (blue) and EthD-1 (red). (B) Percentage of Live and Dead cells counted using ImageJ. (C) Immunostaining for DAPI, F-actin, Ki-67, and Collagen IV after NIH-3T3 cells encapsulation in hydrogels for 1 and 15 days.

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

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Three-dimensional (3D) *in vitro* tumor models are important for recreating the complexity of the tumor microenvironment (TME) and testing new anti-cancer drugs. Conventional 2D *in vitro* models and animal models are not capable to mimic the human tumor biology. 2D models lack of complex intracellular interactions occurring *in vivo* whereas animal models have different metabolic pathways and drug response and toxicity. 3D tumor models can solve these limitations as they can recreate cell- cell and cell-extracellullar matrix (ECM) interactions, production of ECM, hypoxia, gradients of pH, O2 and nutrients of human tumors. ECM is the main component of the TME and is involved in cancer progression [1]. Cell-derived matrices (CDMs) can mimic the complexity of the tumor ECM [2]. Our aim is to fabricate CDMs from fibroblast, that can be used to obtain more physiologically relevant cancer models.

In this study, we cultured human dermal fibroblasts (hDFs) with microparticles (MPs) for the production of *in vitro* 3D tumor models. Polylactic acid (PLA) MPs were produced by jet break-up method and then functionalised with fibronectin. Afterwards, hDFs were seeded on the MPs with a spinner flask and leaved in culture for 10-15 days to produce CDM. Before 3D experiments, the optimal conditions to obtain CDM were optimized using conventional 2D cell culture. hDFs were treated with TGF□-1 to differentiate fibroblasts into myofibroblasts and ascorbic acid to induce collagen synthesis. Also, the effect of Ficoll 70/400, a macromolecular crowder, and starvation were assayed. The cellular viability and cell seeding in the MPs were evaluated. The ECM production was evaluated by BCA, hydroxyproline, SEM and immunofluorescence. Values were normalized by the total DNA.

Based on our results, Ficoll, TGF□-1 and ascorbic acid combination increased total protein and total collagen production in 2D culture for 10 days in contrast to the control group. Moreover, the ratio of □- SMA positive cells was higher when hDF cells which were treated with TGF□-1 for 7 days, suggesting their differentiation into myofibroblasts.

In conclusion, CDMs are promising biomaterials for establishing 3D *in vitro* tumor models for cancer research, testing anti-cancer drugs and for personalized medicine in the coming years.

ACKNOWLEDGEMENTS

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Development of a bioprinted breast cancer model using tissue derived extracellular matrix

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3D bioprinting has emerged as a promising technology for fabricating artificial tumors as it allows the fabrication of complex models recreating tumor physiology. The importance of the extracellular matrix (ECM) in tumor progression and drug resistances, has motivated the development of more biomimetic tumor-ECM bioinks.[1] Decellularized tissues-derived matrices (TDMs) can provide the native breast tissue biological cues, but its inadequate mechanical properties prevent their bioprinting. The aim of this work is to develop a breast TDM-like bioink suitable for bioprinting breast cancer models without a sacrificial material.

Porcine breast tissues were decellularized and delipidated, and its composition was studied. TDMs pre- gels were fabricated by digesting it with pepsin and neutralizing the pH. The addition of alginate and gelatin methacrylamide (GelMA) into the bioink was also assayed. TDM bioinks were printed with a 3D bioplotter (RegenHU) and then crosslinked with UV, $CaCl_2$ and temperature. The bioinks were further tuned by incorporating an ECM protein overexpressed in breast cancer, Collagen type 1 (Col1). The shape fidelity of bioink was characterized and the hydrogels Young modulus was measured. For bioprinting artificial breast tumors, cell-laden bioinks were prepared by dispersing breast cancer cells (BCCs) or mesenchymal stemcells in the bioink. Cellular survival, proliferation, morphology, and the expression of adhesion molecules were studied. The bioprinted hydrogels were used to study the efficacy of doxorubicin (Dox).

The addition of GelMA and alginate allowed the TDM bioprinting. BCCs proliferate in TDM bioinks and form spheroids. Col1 addition improves the bioink printability, increases cellular proliferation and reduces doxorubicin sensitivity. TDM bioinks allow BCCs and stromal cells bioprinting and could be used to fabricate artificial tumors. Taken together, we have proved that TDM bioinks could be used for bioprinting artificial breast tumors closely recreating the tumor ECM.

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Easily applicable modifications to electroporation conditions improve the transformation efficiency rates for rough morphotypes of fast-growing mycobacteria

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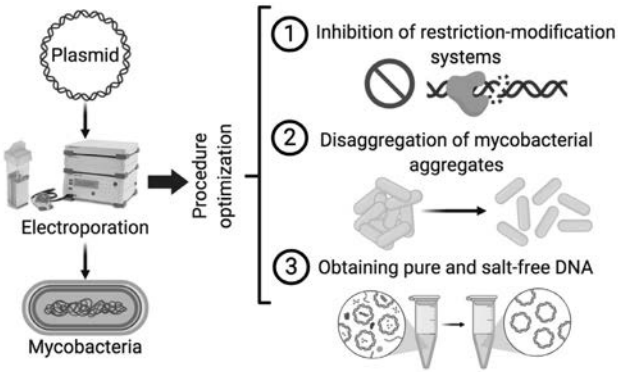
Electroporation is the most widely used and efficient method to transform mycobacteria. Through this technique, fast- and slow-growing mycobacteria with smooth and rough morphotypes have been successfully transformed. However, transformation efficiencies differ widely between species and strains. In this study, the smooth and rough morphotypes of *Mycobacteroides abscessus* and *Mycolicibacterium brumae* were used to improve current electroporation procedures for fast-growing rough mycobacteria. The focus was on minimizing three well-known and challenging limitations: the mycobacterial restriction-modification systems, which degrade foreign DNA; clump formation of electrocompetent cells before electroporation; and electrical discharges during pulse delivery, which were reduced by using salt-free DNA solution. Herein, different strategies are presented that successfully address these three limitations and clearly improve the electroporation efficiencies over the current procedures. The results demonstrated that combining the developed strategies during electroporation is highly recommended for the transformation of fast-growing rough mycobacteria.



Graphical abstract. New Biotechnology. 2021 Jul 25; 63: 10-18. Epub 2021 Feb 23. PMID: 33636348. DOI: 10.1016/j.nbt.2021.02.003

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Collagen-tannic Acid Spheroids For β-cell Encapsulation Fabricated By 3D Bioprinting

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Type 1 Diabetes (T1DM) results from autoimmune destruction of pancreatic insulin-producing β-cells. Nowadays, insulin injections remain the leading therapeutic option. However, injection treatment fails to emulate the highly dynamic insulin release that β-cells provide. During the last years, 3D cell-laden microspheres have been proposed as a major platform for bioengineering insulin-secreting constructs for tissue graft implantation and a model for *in vitro* drug screening platforms.

Current microsphere fabrication technologies have several drawbacks: the need for an oil phase containing surfactants, diameter inconsistency of the microspheres, and high time-consuming processes. These technologies have widely used alginate for its rapid gelation, high processability, and low cost. However, its low biocompatible properties do not provide effective cell attachment. To overcome these limitations, this study proposes a high-throughput 3D bioprinting methodology that employs an ECM-like microenvironment for effective cell-laden microsphere production. Crosslinking the resulting microspheres with tannic acid (TA) prevents collagenase degradation and enhances spherical structural consistency while allowing the diffusion of nutrients and oxygen. In addition, the approach allows customization of microsphere diameter with extremely low variability. In conclusion, we developed a novel bio-printing procedure to fabricate large amounts of reproducible microspheres capable of secreting insulin in response to extracellular glucose stimuli.

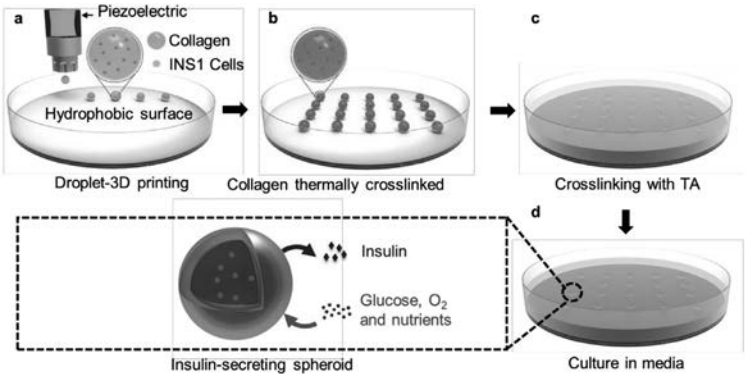

Figure 1. General overview of fabricating INS-1E-laden collagen scaffold crosslinked with TA.

Biomaterials for retina epithelial cell transplantation as treatment for degenerative macular disease

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Currently, no proven treatment options exist for patients with geographic atrophy, an advanced form of age-related macular degeneration (AMD). For selected patients with extensive drusen or geographic atrophy, cell transplants might prevent central vision loss through replacement of dysfunctional or dead retinal pigment epithelium (RPE) cells. To date, several studies have explored subretinal delivery of stem cell–derived RPE cells in AMD patients: An ESC-derived RPE cell suspension was tested in a phase 1 clinical trial of patients with GA (dry) stage of AMD; an autologous iPSC-RPE (iRPE) sheet was recently transplanted in one patient with the wet form of AMD; and ESC-derived RPE patch on a paralene scaffold was tested in four dry AMD patients, and a similar patch on polyester scaffold was tested in two wet AMD patients. These landmark studies in the field of regenerative medicine demonstrated the safety of various cell replacement therapies. A major obstacle to the success of RPE transplants in AMD patients is the failure of transplanted RPE cells to survive and become functional in the diseased AMD eye. In our study, we fabricated a patch of human methacrylated collagen Type 1 (Col-Met) for tissue-specific adult human RPE stem cells delivery and survival. First, we have tuned the mechanical properties of Col-Met using two photo-initiators (Ruthenium and LAP) and time-exposure to the UV. The Col-Met crosslinked with Ruthenium is stiffer than LAP and lesser prone to the degradation to collagenase. After that, we investigated the way the cells interact with material: encapsulated or seeded on top of Col-Met. Assays of viability and cell morphology demonstrated that cells seeded on top of Col-Met allow the formation of native RPE monolayer. Furthermore, the ultrastructural analyses (SEM and TEM) of the patches revealed that the RPE monolayer is anchored to the Col-Met allowing both the increase of cell pigmentation and synthesis of primary cilium. The next step was focused on mixing the Col-Met with different laminins (532, 332, and 111) to see what Col-Met-laminin combination resulted in a closer native environment for the cells. The laminin 332 increased the cell proliferation pigmentation of RPE as demonstrated by confocal microscopy and q-PCR. In conclusion, Col-Met-LAP represents a promising approach for RPE stem cells transplantation allowing to tune its degradability once the cells attached and proliferate.

Stromal SMAD3 enhances cancer cell 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 step s 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- ?1 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 form 3D tumor spheroids by mixing fluorescently labelled lung cancer cells and fibroblasts (1:2) within a gel containing type I collagen mixed with Matrigel analyze by confocal microscopy. Our preliminary results revealed that, after 48h and in the absence of exogenous TGF-?1, we observed more invasive branches and a higher length in tumor spheroids containing shSMAD2 fibroblasts compared to shSMAD3 fibroblasts. 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-?1) what is observed in ADC-TAFs and may contribute to the early dissemination that is frequently observed in the clinic.

Fatty hepatocytes induce skeletal muscle atrophy *in vitro*: a new 3D platform to study the protective effect of albumin in Non-Alcoholic Fatty Liver Disease

Francesco De Chiara & Ainhoa Ferret-Miñana, Juan M. Fernández-Costa, Alice Senni, Rajiv Jalan and Javier Ramón-Azcón

Non-alcoholic fatty liver disease (NAFLD), as the new silent disease of the 21st century, affects 1 in 4 people worldwide. It ranges from simple steatosis to non-alcoholic steatohepatitis, which may progress to cirrhosis, eventually leading to hepatocellular carcinoma. Many evidences in subjects with NAFLD show that the liver damage propagates to the skeletal muscle tissue leading to loss of skeletal muscle mass and/or physical performance, known as sarcopenia. Why fatty liver influences the development of sarcopenia in NAFLD is still not completely elucidated. Infusion of human serum albumin (HSA) has been demonstrated to reduce renal dysfunction, hospital readmissions and mortality in patients with acutely decompensated cirrhosis. HSA is synthesized in the liver and continuously secreted into the bloodstream, where it is the most abundant protein. It is the main transporter fatty acids and plays major roles in the binding of drugs and free radicals. In a previous work, we have demonstrated that hepatocytes cultured *in vitro* release albumin in response to lipids challenge for 48 hours (400 μM non-esterified fatty acids). Our hypothesis is that in NAFLD patients, the fatty liver induces gradual atrophy of skeletal muscle tissues accelerating the progression of liver disease that might be mitigated by administration of albumin. To test this hypothesis, in our laboratory we developed a 3D platform for hepatocytes and skeletal muscle cells crosstalk under non-esterified fatty acids (NEFAs) regimen. In this project, liver AML12 cells and skeletal muscle C2C12 cells were encapsulated in a solution of gelatine methacryloyl and carboxymethylcellulose at concentration of 5% and 1%, respectively. The photo-initiator LAP was then added at concentration of 0.1% and the polymer exposed at UV light for 30 seconds. The 3D tissues were fabricated using PDMS as moulds. 3D hepatocytes showed the typical signs of NAFLD such as lipid accumulation, metabolic activity impairment and apoptosis after 72h of culture with NEFAs mix. The 3D skeletal muscle cells incubated with supernatant from fatty hepatocytes displayed loss of cytoplasmatic mass, impaired metabolic activity, and altered genes expression involved in the maturation of myotubes. In the following set of experiments, we pre-treated healthy hepatocytes with albumin prior to incubation with NEFAs, then we collected the supernatant and treated the skeletal muscle cells. We have seen reduced hepatocytes' lipids accumulation and signs of cell death, lower level of ammonia in the supernatant and improved muscle mass in the skeletal muscle cells. In conclusion, this study establishes the direct connection between liver and skeletal muscle during the development of NAFLD, and the beneficial effect of albumin treatment on both liver and skeletal muscle tissue in an *in vitro* model of NAFLD. The tool herein presented can be employed as a customizable 3D *in vitro* platform for drug screening.

Skeletal muscle-based 3D-bioengineered actuators as platforms of biomedical interest

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Recent advances in three-dimensional (3D) bioprinting and tissue engineering have opened new possibilities in the fabrication of bioengineered muscle models to mimic the complex hierarchical organization and functional properties from the native tissues. The combination of skeletal muscle tissue and artificial elements has led to a wide variety of innovative solutions for the creation of bio-hybrid robotics [1] and bioactuators [2]. The fabrication of a biomimetic muscle tissue by using 3D bioprinting is key to better understand the physiological conditions of native muscle in order to integrate some of their unique properties to the bioactuator platform, such as self-healing, adaptability, or response to external stimuli. Moreover, these muscle-based bioactuators offer the opportunity to study other processes of interest in the biomedical field, such as muscle development, regeneration, and diseases in a biomimetic environment that resembles the native tissue.

In collaboration with Lubrizol, we developed a 3D-printed bioengineered human skeletal muscle platform to obtain both a healthy and an aged tissue model or relevance for drug testing [3]. To induce the morphological and functional aging conditions, the bioengineered muscle tissues were treated with tumor necrosis factor [?]. Furthermore, we evaluated the effects of Argireline® Amplified peptide as a cosmetic agent that causes muscle relaxation. Moreover, to obtain the bundle-like structure present in the native muscle, we explored an alternative co-axial 3D printing approach to create thinner fibers that can be hierarchically organized. The resulting cell-laden scaffold shows an improved maturation process due to an enhanced nutrients diffusion during the differentiation process, presenting an increased force output when a higher number of fibers are included in the 3D cell construct.

After exploring the potential of 3D printing for fabricating 3D bioengineering platforms for biomedical applications, our interests are currently focused on exploiting the regenerative capabilities of muscle tissue to integrate self-healing properties to living actuators [4] and create more biomimetic *in vitro* muscle models.

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Generation of reporter human pluripotent stem cell lines to study cardiac and renal development and disease

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The development and use of human pluripotent stem cells (hPSCs), represent an effective tool to recapitulate characteristics related to human disease, tissue differentiation and morphogenesis. This is due to their capacity to generate three dimensional (3D) self-organized organ-like structures, called organoids, that can constitute an effective alternative to animal models. Until recently, the genome of hPSCs was difficult to manipulate, limiting considerably their use for functional genetics. Thanks to the development of the CRISPR-Cas9 technology, it is now possible to enable precise, intentional, and permanent changes in the DNA of living organisms and cells. Our laboratory has developed a cellular platform, named iCRISPR2 (iC2), that allows to perform highly efficient genome editing in hPSCs, through TALEN-mediated gene targeting that introduces a doxycycline-inducible Cas9 expression cassette at the AAVS1 locus. This editing platform enables a myriad of applications such as the generation of reporter, knock- out and knock-in cell lines. We focus our attention on the generation of cardiac and renal reporter cell lines. To this aim we designed highly active and specific sgRNAs, to make Cas9-mediated site-specific double strand breaks (DSB). Each specific sgRNA was co-nucleofected in iC2 line, together with a specific double strand DNA donor sequence that matches the broken ends of the genomic DNA. In the middle section of the donor DNA we introduce the sequence of a specific reporter gene. After the Cas9 mediated double strain break, the DNA repair machinery catalyzes the homology-directed repair (HDR), using the donor DNA as template to fill the gap. As consequence of this repair event, the sequence of the reporter gene is introduced into the genome. Nucleofected cells are plated as single cells and cultivated until clones appear. Clonal lines are established by manual colony picking. By genotyping analysis, we identified positive clones, in which after the DSB, the DNA repair machinery introduces the sequence of the reporter gene in the genome. Following this strategy we have been able to start generating cardiac (MYH6, MYL2, SIRPA) and renal reporter cell lines (MEIS-1 and GATA3) for applications on organoid derivation. Currently, our efforts are guided towards the definition of cell culture conditions sustaining cardiac and renal differentiation exploiting these cell sources. Combining these reporter cell lines with cell culture conditions mirroring systemic conditions compromising organ function already available in the laboratory (i.e., Diabetes) will lead to the generation of advanced biomodels to study cardiac and kidney diabetic disease.

Study of epigenetic modifications related to the cellular prion protein expression as preclinical biomarkers of Alzheimer's disease

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There have been great advances in the diagnosis of Alzheimer's disease due to the correlation between imaging tests, such as PET, for classic biomarkers (A? peptide and hyperphosphorylated tau) and their concentration in cerebrospinal fluid or even in peripheral blood. Tracking these markers is a good prognostic indicator, since brain damage is directly related to the accumulation and spread of amyloid stools, mainly from the tau neurofibrillary tangles. However, we are still far from being able to screen the population in asymptomatic stages of the disease, to intervene and prevent pathological progression. This occurs because affected people come to ask for a diagnosis when symptoms of cognitive deficits are coming. Consequently, brain damage is already irreversible.

According to the pathological anatomical study of human post-mortem brain tissue, there is an increase of cellular prion protein (PrPC) expression in the asymptomatic stages of the disease (histopathologically characterized samples at initial stages of Braak (from I to II))[1, 2]. In this sense, PRNP transcriptional activity responds to tau levels between other factors, being AP-1 site the most functional promoter regulation region[3]. In addition, correlation of PrPC levels between brain and plasma has already been demonstrated[4]. And, correlation between blood and brain epigenetic profile has been reported in Alzheimer disease (AD)[5].

Altogether, we aim to analyse epigenetic markers related to PrPC expression changes as future candidates for detection in peripheral blood in AD asymptomatic population. Using brain human samples from diagnosed AD patients at different Braak stages (from I to VI) our results showed an increase of H3K9 histone acetylation associated with PRNP promoter in the first steps of the disease when compared to non-AD control samples and, a decrease at later stages, correlating with PrPC expression profile. On the other hand, the finding of a methylated cytosine near the AP-1 site in samples from patients with advanced AD, points this mechanism of regulation of PRNP transcription as a candidate to be evaluated in the progression of the disease.

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3D Engineered living systems as swimming robots

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Biohybrid robotic systems are designed based on the combination of synthetic materials and biological entities aiming to acquire improved performance or properties that are difficult to mimic with artificial materials [1]. The integration of biological components (i.e. skeletal muscle cells) in robotic systems provides some of the desired capabilities from such living entities, including linear contraction, self- organization, self-healing, energy efficiency, high power-to-weight ratio, adaptability, or bio-sensing [2]. Three-dimensional (3D) printing is a versatile technique that has opened new possibilities for the fabrication of bio-hybrid robots, permitting the easy integration of 3D structured biological and synthetic elements to obtain advanced actuation modes.

In this work, we develop a skeletal muscle-based swimming biobot formed by a 3D-printed serpentine spring integrated into a 3D cell laden scaffold [3]. The spring skeleton provides dynamic mechanical self-stimulation during the cell differentiation process, promoting a greater maturation and alignment of the muscle fibers that results in a higher force output. Upon electrical stimulation, the biobot exerts a directional swimming motion at the liquid-air interface. We achieved a maximum velocity of 800 μm/s, 791 orders of magnitude higher than the fastest skeletal muscle-based swimming biobot up to date. Additionally, alternative skeletal-muscle based biobot configurations arising from a self-assembly process of the 3D cell-laden scaffold are currently under study. They present a characteristic infinity shape that results in a crawling motion by either spontaneous contraction or controlled electrical pulse stimulation. The infinity biobots guidance has also been explored by using topographical and external magnetic fields. Both the self-stimulating skeleton and the self-assembly process demonstrated in our studies are of great interest to the 3D bioengineering robotic community, providing useful tools to create advanced robotic systems with programmable actuation.

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Cryopreservation of 3D cell culture models

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Cryopreservation methods for cell and tissue storage have been around since 1954, where thawed sperm samples were used for an insemination[1]. Since then, the technology has evolved for clinicians and researchers to cryopreserve tissue, cell lines and primary cells. While cryopreserving tissues helps maintain their physiological integrity for study it does not assure the viability of the cells after thawing. Furthermore, the cells cryopreserved in suspension lose their dimensional anchors, forcing them to change their morphology and influence their function after thawing[2].

To solve this issue, tissue engineering allows researchers to create 3D culture models, such as organoids and bioprinted cell clusters, that mimic the physiological characteristics of the cells in tissue and disease[3]. Although this culture methods present promising results, there is a lack of methodology to cryopreserve 3D cell models and patients' samples for storage and transport in a way where they remain viable and structurally sound after thawing. As a first step to overcome this challenge we propose a protocol that uses a carboxymethyl cellulose scaffold and precise freezing and thawing conditions for spheroid vitrification, cryopreservation, and survival. We have tested this method with hepatocytes. The scaffold provides structure for the hepatocytes to create spheroids on their own before the procedure as well as support throughout the freezing and thawing processes for optimal cell viability post-thawing. We have observed that this method achieves higher cell viability than transporting the cells as a cryopreserved pellet for model assembling after thawing by allowing the cells to settle and form a pseudo-tissue beforehand to improve viability and cell function after cryopreservation. This technique constitutes a step forward in the cryobiology field for it will facilitate the transport of already assembled 3D models from cell lines or primary cells from patients to the research and drug testing facilities with high viability and model integrity after assembly.

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Organ-on-a-chip System to Study Biomaterial-induced Vasculogenesis

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Over the last decades, most of the strategies to improve the vascularization of biomaterials based on calcium phosphates (CaPs) were 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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Cannabinoid 1 receptor stimulation increases the regenerative capacity of sensory neurons

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In the last years, many studies have highlighted the importance of boosting the intrinsic regenerative capacity of neurons after axonal injury. In that sense, cannabinoid compounds have been shown to be important in neuroprotection and neuromodulation after CNS injuries. However, their effects on axon regeneration remain unknown. Cannabinoids exert their function acting on the endocannabinoid system (ECS), which it is composed of cannabinoid receptors, including the cannabinoid type-1 receptor (CB1R) which is highly expressed in neurons; their endogenous ligands, and the enzymes involved in their synthesis and inactivation.

Here, we have studied the effects on regenerative capacity in neurons from the dorsal root ganglia (DRG) after ECS modulation.

We have observed that increasing the endocannabinoid tone, through an inhibition of an endocannabinoid degradative enzyme, increases neurite outgrowth in DRG neurons *in vitro*, through neuronal CB1R activation. In fact, the CB1R agonist ACEA also enhances neurite outgrowth in cultured DRG neurons, by activating the PI3K signalling pathway. Additionally, ACEA also improves axon growth *in vivo* in mice after sciatic nerve crush.

Interestingly, we also measured axon outgrowth in cultured DRGs after a conditioning sciatic nerve lesion combined with rimonabant, a CB1R antagonist. We found that neurite outgrowth induced by a conditioning lesion was blocked by rimonabant administration, suggesting that CB1R is involved in the conditioning injury-dependent axonal regeneration.

Altogether, these results highlight the importance of the ECs in promoting the intrinsic regenerative capacity of sensory neurons after injury and opens new therapeutic windows to treat axonal injuries.

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Study and analysis of class la ribonucleotide reductase from *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a versatile opportunistic pathogen that easily adapts to changing environments. For that reason, and along with increasing antimicrobial resistance, there is an urgent need to find new therapies to combat these infections. Ribonucleotide reductases (RNRs), a family of metalloenzymes involved in dNTP synthesis, is a key enzyme for life and represents a suitable target to fight this microorganism. Class la RNR of *P. aeruginosa* is encoded by the *nrdAB* operon, regulated allosterically and transcriptionally by NrdR, AlgR, and DnaA. However, nowadays, there are still some gaps in its regulation.

It has been described a long 5'UTR (untranslated region) on nrdA mRNA, and it is reasonable to think that this 5'UTR region plays a key role in *nrdA* regulation.

Bioinformatic analysis suggested that 5'UTR could be a cobalamin riboswitch and/or an small RNA (sRNA). Experimental assays do not confirm this first hypothesis. However, the second hypothesis was verified by RT-PCR. It is known that sRNAs have plenty of functionalities, the most noteworthy are mRNA stability and half-life. Via a shut-off transcriptional assay was proved that 5'UTR decreased *nrdA* mRNA half-life.

Nevertheless, several experiments should be performed to exclude or confirm one or both hypotheses, apart from additional tests and further bioinformatics analysis.

Keywords: *Pseudomonas aeruginosa, antimicrobial resistances, ribonucleotide reductases, class la RNR, nrdAB, UTR, cobalamin riboswitch, sRNA, mRNA stability, half-life.*

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Neuronal activity effects on axonal growth after spinal cord injury

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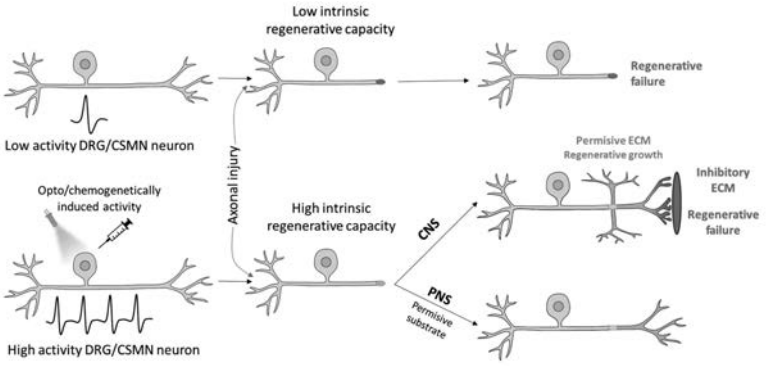
Spinal cord injuries (SCI) frequently lead to permanent disabilities as a consequence of the existence of both extrinsic and intrinsic axon growth barriers in the central nervous system (CNS). For proper restoration both barriers need to be overcome. In terms of patient treatment, activity-based therapies, which are the current leading strategies for recovery, are showing limited improvements, but their underlying molecular bases remain unknown.

Sustained neuronal activity stimulation with chemogenetics prior to peripheral nerve injury (sciatic nerve crush; SNC) resulted in enhanced axon growth. However, increasing neuronal activity in the corticospinal tract or the dorsal columns after CNS injury (SCI) did not result in behavioral improvements. Histologic analysis showed increased sprouting in white matter above injury, but not below the injury level. These findings seem to indicate that even tough neuronal activity reactivates the growth capacity of neurons, this capacity is not sufficient to overcome the extrinsic barrier generated after injury in the CNS. Thus, for translational purposes, this strategy would need to be combined with management of the inhibitory scar in order to promote long distance, functional regeneration.



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Novel Method for the Quantification of Tissue Decellularization

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Tissue decellularization is typically assessed through absorbance-based DNA quantification after tissue digestion. This method typically consists of the digestion of a piece of decellularized tissue, followed by the DNA purification using one of many commercially available DNA quantification kits. The remaining DNA is then quantified with a spectrophotometer: if the DNA concentration is below a certain threshold, the sample is considered to be decellularized. This method has several disadvantages, namely its destructive nature and inadequacy in experimental situations where tissue is scarce. Here, we present an image processing algorithm for quantitative analysis of DNA content in (de) cellularized tissues as a faster, simpler and more comprehensive alternative. Our method uses local entropy measurements of a phase contrast image to create a mask, which is then applied to corresponding nuclei labelled (UV) images to extract average fluorescence intensities as an estimate of DNA content. By using entropy, uneven lighting does not pose a problem when performing image segmentation. The method can be used on native or decellularized tissue to quantify DNA content, thus allowing quantitative assessment of decellularization procedures. We confirm that our new method yields results in line with those obtained using the standard DNA quantification method and that it is successful for multiple types of tissues, such as lung, heart, bladder, and kidney. We are also able to accurately obtain a timeline of decreasing DNA signal with increased incubation time with a decellularizing agent. This decreasing DNA signal obtained with the novel image-based method followed the same trend of decreasing DNA content measured by DNA quantification of digested lung samples. Finally, the identified masks can also be applied to additional fluorescence images of immunostained proteins such as collagen or elastin, thus allowing further image-based tissue characterization and providing additional information on the effects of the decellularization procedure on the matrix.

Dissecting early nephron patterning and morphogenesis in kidney organoids derived from human pluripotent stem cells

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INTRODUCTION. The kidney develops from the ureteric bud progenitor cells (UB) and the metanephric mesenchyme (MM). The UB extends into the MM and branches repeatedly to give rise to the collecting duct system, whereas the MM undergoes mesenchymal to epithelial transition (MET) to generate epithelial renal vesicles (RVs), that develop into nephrons through patterning and segmentation (nephron induction). Kidney developmental studies performed in the mouse have shown that nephron patterning is guided by Wnt/□-catenin and Notch signalling pathways leading to the specification of the different nephron segments (proximal, medial and distal). Moreover, tissue formation and multi-cellular morphogenesis related programs are also influenced by intrinsic tissue mechanics. Still, how developmental signalling pathways work in concert with tissue-scale morphogenetic and biomechanical changes to guide nephron formation remain unknown. Here, we propose the use of kidney organoids from human pluripotent stem cells (hPSC) as an unprecedented human *in vitro* model to provide a mechanistic understanding of early steps of nephron formation. We have recently shown that Wnt/□-catenin and Notch signalling pathways are autonomously recapitulated *in vitro* during nephron formation and patterning in hPSC-kidney organoids. Moreover, that tightly controlling cell-cell and cell-ECM interactions results in the generation of higher-grade kidney organoids, and that the use of soft hydrogel substrates accelerates hPSC-kidney organoid development.

OBJECTIVE. We next aim to identify, dissect and perturb the emergent multicellular behaviours that occur during renal induction and nephron formation (namely, patterning and segmentation) in hPSC-kidney organoids.

METHODS. We have set up a system to drive the differentiation of nephron progenitor cells (NPCs) from hPSCs towards RVs and their further transition into nephron-like structures on compliant polyacrylamide hydrogel substrates with controlled stiffness in a 2D culture setting. Polyacrylamide hydrogels of 1 kPa (soft) and 60 kPa (rigid) are generated by adapting the compositional ratio of acrylamide to bis-acrylamide and are further functionalized using acrylic acid NHS. Using this system, we aim to spatiotemporally characterise early steps

of nephrogenesis from hPSCs by immunofluorescence and confocal analysis, time-lapse imaging and traction force microscopy (TFM). These analyses will be performed at different stages during renal differentiation including nephron progenitor cells (NPCs) induction, RV emergence, proximal-distal RV polarization and formation of the nephron-like segments.

CONCLUSIONS. Our methodology offers the opportunity to qualitatively and quantitatively monitor emergent multicellular behaviours during hPSCs renal induction and nephrogenesis in microenvironments with controlled rigidity. Our preliminary data suggests that soft substrates facilitate the generation of nephron-like structures. Next, this hPSC-kidney organoid 2D-setting will be used to study how molecular and mechanical cues impact cell-cell and cell-ECM interactions via feedback mechanisms to guide the shaping (patterning and segmentation) of the nephron.

3D Brain-on-a-chip for Organoptypic Culture and Differentiation of Neuroprogenitor Cells

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Current 2D *in vitro* and animal models are not sufficient to fully understand the brain and its diseases mainly because of their lack of complexity, ethical concerns, and differences in species. New engineered models, such as 3D culture or microfluidic devices, have been proved to be interesting choices for better resembling the brain[1]. This work aims to mimic the brain microenvironment with a 3D microfluidic device to study the differentiation process of neuroprogenitors cells. The scaffold material used for 3D culture is composed of a combination of different polymers (methacrylated gelatin, methacrylated alginate, and hyaluronic acid). The physical characterization of the biomaterial was performed and results showed that this material has a good water intake and a low compressive Young's Modulus, close to the values of the native tissue. Neuroprogenitor mouse cells were embedded in the hydrogel and placed in the microfluidic device for up to 8 days. The viability and differentiation into neurons were tested through a LIVE/DEAD assay and immunostaining. Cells in Matrigel were used as control. Results showed high cellular viability, proliferation, and differentiation into early neurons, with increased connectivity between cells, proving that this model can be used as a platform with human cells for future studies on neurodegenerative diseases.

Keywords: *3D models, neuronal culture, microfluidic devices, biomaterials, hydrogels.*

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Personalized Biodegradable 3D Printed Implant for Guided Bone Regeneration

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Metallic meshes for guided bone regeneration (GBR) to treat maxillary bone defects present some relevant benefits, such as giving graft stability and preservation. Although these advantages, there are still some important drawbacks, as using an autologous bone graft, the need for a second surgery to extract the mesh after finishing the treatment, or mesh exposure prevalence. The aim of this project is to develop a biodegradable and bioactive personalized implant to substitute the use of metallic meshes. This implant will stimulate osteogenic and angiogenic processes, as well as avoiding a second surgery to remove the metallic mesh.

Polycaprolactone (PCL) was used in combination with different calcium phosphate-based bioactive microparticles (MPs). MPs were dispersed into PCL and extruded by 3D printing. *In vitro* cell characterization was studied using human mesenchymal stem cells (hMSCs) and human gingival fibroblasts (hGFib) seeded on the scaffolds. *In vivo* subcutaneous scaffolds implantation was evaluated using 8 weeks old CD1 mice. The possibility to obtain implants with personalized complex geometries was assessed by using polyamide defect models from clinical cases, kindly provided by AVINENT® Implant System.

Macroporous 3D printed scaffolds were successfully fabricated with homogeneous MPs dispersion into PCL according to FESEM and µCT images. Scaffolds remained stable under simulated physiological conditions for one year, with minimum weight loss. Cytotoxicity was not detected for all scaffolds tested evidencing a good biocompatible behavior as indicated by cell metabolic assays. Fluorescent confocal images indicated that scaffolds were fully colonized by hGFib, showing good cell attachment that would decrease the risk of implant exposition. Moreover, the biocompatibility observed *in vitro* studies was confirmed *in vivo* as well. Subcutaneously implanted scaffolds in mice for two months showed tissue and vessel infiltration. Finally, implants from clinical cases with complex geometries have been successfully printed and tested on polyamide models presenting a proper fit to the defect.

A promising implant to substitute the use of metallic meshes for GBR is being developed, not only maintaining their benefits but also solving their main side effects. Further experiments are ongoing to continue to study the implant bioactivity and select the most efficient condition.

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Gelatin-based hydrogels compatible with neuronal culture and differentiation

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3D *in vitro* models resemble more accurately the physiological conditions of the brain [1]. However, neuronal cultures face a challenge due to their high sensitivity to changes in their surroundings [2]. We present in this work a hydrogel composed by methacrylated gelatin (GelMA), alginate (AlgMA) and hyaluronic acid (HA) for neural progenitor cell culture. Our goal was to assess the compatibility of neuronal culture in GelMA and AlgMA composites, since these two materials have been used in several tissue engineering applications [3]. HA was added to better mimic the properties of brain tissue. We performed the physical characterization of formulations as well as viability studies with neuroprogenitor cells and *in vitro* differentiation from 8 up to 28 days. Results showed that all hydrogels have good porosity, allowing nutrient and oxygen diffusion. They also present low Young Modulus, especially for hyaluronic acid formulation, rendering values similar to brain tissue. Comparing these new materials with Matrigel, only for 8 days, our formulations allowed neuronal proliferation and differentiation, rendering high viability rates. After 28 days, particularly the hydrogels in culture presented minimal degradation and cells showed projection growth, great connectivity and expression of neuronal markers. Overall, our formulations allow long-term culture, making these hydrogels a promising scaffold for induced pluripotent stem cells long-term culture and differentiation.

Keywords: *3D in vitro models, neuronal culture, hydrogels*

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Establishment of an *in vitro* biomimetic model for the study of human neuromuscular diseases

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Nowadays, *in vitro* models of skeletal muscle are gaining relevance as valuable tools to study the underlying mechanisms of human neuromuscular diseases. Lately, the use of 3D cultures and human cell lines has made these systems increasingly similar to *in vivo* conditions. These models have the ability to recapitulate human muscle differentiation and function, and have the advantage of being simple, scalable, and reproducible. Also, they offer the possibility of developing personalized versions by using patient cell lines and other patient samples.

Similar to recently published protocols, our model uses immortalized human myoblasts cultured in a hydrogel placed between two anchor points, which allows the cells to form an aligned and compacted structure similar to human muscle. After differentiation, the resulting muscle bundle shows contractile activity in response to electrical pulse stimulation or addition of acetylcholine. Additionally, cells show multinucleated morphology, sarcomeric pattern of myosin heavy chain, and formation of acetylcholine receptor clusters (motor end plates). Once the culture of the muscle bundle has been optimized, we are working towards the introduction of human neuronal cells which can form functional connections with the motor end plates. With this setup, we plan to study how disease conditions might affect neuromuscular function in terms of contractile ability.

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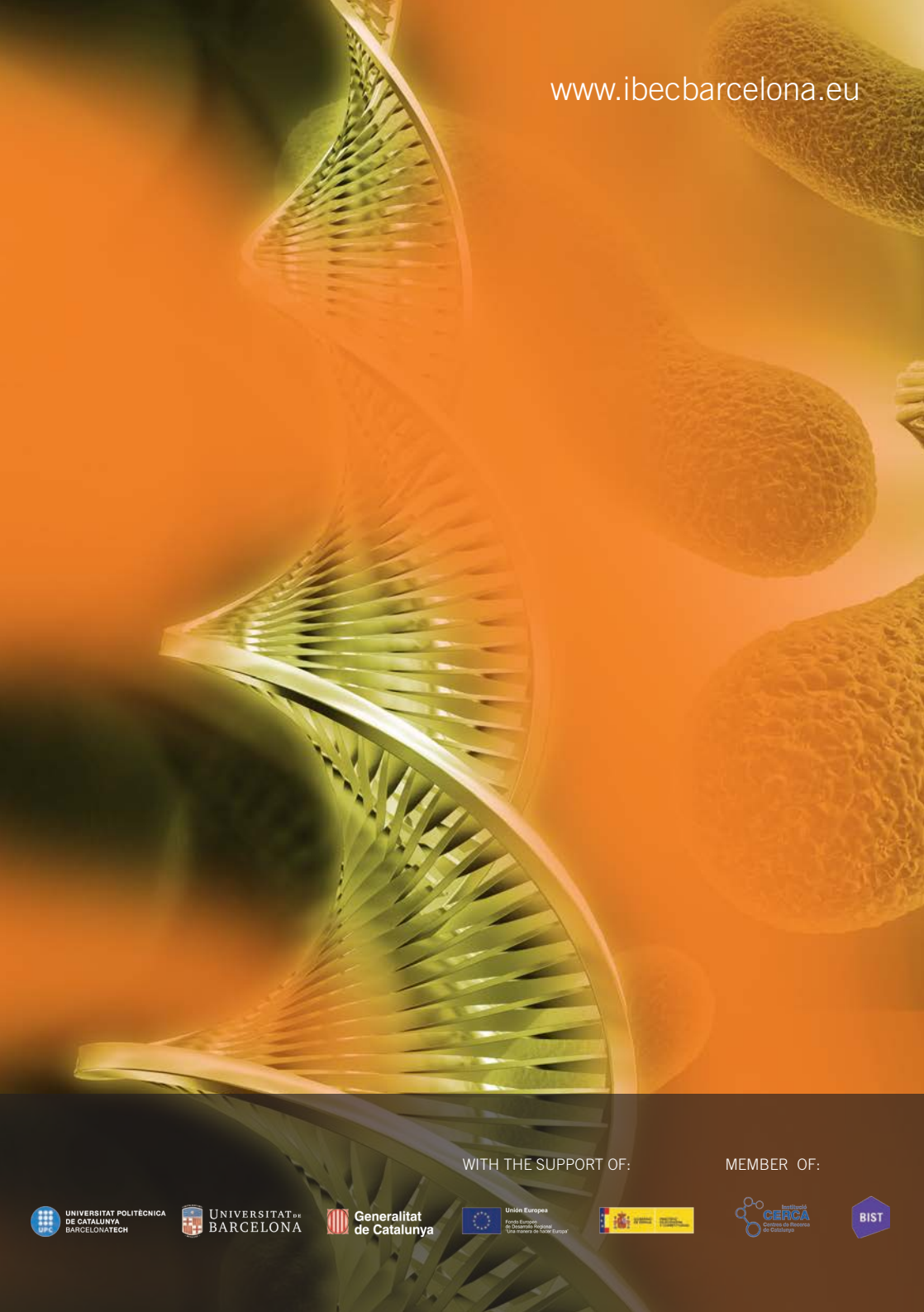

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