FoReCaST

Forefront Research in 3D Disease Cancer Models as In vitro Screening Technologies

HALLMARKS IN CANCER AND THE DEVELOPMENT OF DIAGNOSTIC TOOLS

3rd Workshop

14 - 16 June > Braga > Portugal

FoReCaST

Forefront Research in 3D Disease Cancer Models as in vitro Screening Technologies

WORKSHOP HIGHLIGHTS

The FoReCaST workshop will take place from 14th to 16th of June 2021, in the historical city of Braga, in Portugal.

FoReCaST is the 3rd workshop organized by the 3B's Research Group from the University of Minho (UMINHO) focusing on the *hallmarks in cancer and the development of diagnostic tools*. A range of world scientific leaders will be meeting and presenting their latest research, covering the most relevant topics related to novel diagnostic/screening technologies and tumour models, and their uses in cancer research and drug screening. In this context, 3B's-UMINHO will strategically use the knowledge gained during the last ~22 years in the fields of biomaterials, tissue engineering and regenerative medicine to consolidate this new research topic.

Some of the objectives of this workshop are to allow:

- The discussion of scientific and technological updates as well as new methodologies in this field;
- The development of cooperation platforms to enhance the research and share of knowledge;
- The anticipation of emergent technologies with scientific and technological impact;

All these aspects are essential to improve the level and quality of the research in the field of cancer research.

Scientific Topics:

- 3D tumour models
- Biomarkers
- Biomaterials
- Biophysical parameters
- Biosensing
- Cancer organoids
- Drug screening
- Extracellular matrix
- Microfluidics
- Nanotechnology
- Organs-on-chip
- Scaffolds & hydrogels
- Tumor spheroids
- Tumor stroma

The conference will be held in **Hotel Vila Galé Collection** in the beautiful city of Braga, considered one of the youngest European cities. Founded by the Romans in 16 *B.C.* and denominated "Bracara Augusta", Braga combines its bimillennial History with youth and refreshing vitality. With more than 2000 years of antiquity, Braga is the oldest Portuguese city and one of the oldest Christian cities in the world.

In summary, a wonderful city provides a unique atmosphere and character and a unique perspective of History, food, and wine. Therefore, besides the cutting-edge scientific program, the conference will foster informal interactions between participants and a delightful experience in this beautiful historical city of Braga.

Looking forward to meeting you in Braga!

Rui L. Reis
The Conference Chair

Subhas C. Kundu
The Conference Chair

SUPPORT

The FoReCaST 3rd workshop would like to sincerely thank the following support:

Universidade do Minho
Instituto de Investigação em Biomateriais, Biodegradáveis e Biomiméticos

ICVS/3B's
Associate Laboratory
University of Minho

Funded by:

FoReCaST, under the Grant Agreement Number 668983 have received funding from the European Union's Horizon 2020 Research and Innovation programme.

GENERAL INFORMATION

All the information contained in this book is accurate at the time of its publication. The Workshop Organizers reserve the right to alter the programme and the associated events as circumstances dictate.

WORKSHOP CHAIRS
Prof. Rui L. Reis
Prof. Subhas C. Kundu

LOCAL ORGANIZING COMMITTEE

Ana Guerra
Banani Kundu
Catarina Abreu
David Caballero
Joaquim M. Oliveira
Luisa Rodrigues
Miguel Neves
Ricardo Pires
Sandra Carvalho
Vitor Correlo

CONGRESS VENUE
The conference will be held in Hotel Vila Galé Collection, Braga, Portugal at D. Afonso Henriques room.

Hotel Vila Galé Collection
Largo Carlos Amarante,
4700-308 Braga
Portugal

Telephone (+351) 253 146 000
Fax (+351) 253 146 049

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COVID-19 RULES

In the current context of responding to the epidemiological situation of the new coronavirus / COVID-19, FoReCaST workshop is being held according to measures, guidelines and recommendations from the competent authorities and entities.

GETTING TO AND FROM THE VENUE
From the south: A1 motorway north, in the direction of Porto » A3 motorway from Oporto to Braga. Follow through the exit Braga Centro.

From the north: A3 motorway from Valença, in the direction of Braga. Follow through the exit Braga Centro.

Once in Braga: Follow through Dom Frei Caetano Brandão Street and Dom Afonso Henriques Street to Largo Carlos Amarante.

For those of you arriving in Braga by train, the Hotel Vila Galé Collection Braga is 1.2 kilometres from the train station, 15min walking.

GPS Coordinates: N 41° 32' 56.139" - W 8° 25' 24.336"

REGISTRATION AND INFORMATION DESK
All attendees must be registered for the workshop. Admission to the workshop is permitted only to those wearing the official workshop badge. If a name badge is misplaced, please contact the registration desk.

Certificate of Attendance will be provided to all registered participants through email after the completion of the workshop.

The information/registration desk will be located at the Foyer of D. Afonso Henriques room in the first day of the conference and will be open during the following days.

INTERNET
Wireless network at Hotel Vila Galé Collection will be available with free access.

LUNCH AND DIETARY REQUIREMENTS
Lunch planned for the conference is included in the registration fee and will be served in the Hotel Vila Galé Collection restaurants Fundação and Bracara Augusta. Please inform Organizing Secretariat at registration desk as soon as possible in case you have any dietary requirements.

SMOKING POLICY
From 1st January 2008 legislation was introduced in Portugal, which makes it forbidden to smoke in all public places. This includes cafes, bars and restaurants (excluding those with signalized smoking areas). Smoking is only allowed outside the conference building.

PHOTOGRAPHY POLICY
Recording and photographing workshop presentations will not be allowed.

ELECTRICITY SUPPLY
220V is the standard power supply throughout Portugal. If you need a plug or a power adapter, you may find in electronic specialty retailers or ask in the registration desk.

TRANSPORTATION
In Braga, there is a bus that lets you travel through the city centre but, as it is not a very big city centre, you probably will prefer to walk by foot and enjoy the harmony of this city.

Renting a car can be a very nice solution, because, in a small city, there is not many traffic jams, and if you want to stay in a place "far" from the centre, it can be a wonderful transport. It's not so expensive to rent a car but, if you want to feel the city, you can not make longer trips, because the city centre has a lot of streets, that you can only enjoy walking.

Airport: www.ana.pt

Train: www.cp.pt

Bus: www.tub.pt

Taxis operate 24 hours and can be ordered from the Event Venue or from your hotel. Taxis can be hailed in the streets if they have the green light on in the front that says “TAXI”. Do not use unlicensed taxis, which are ordinary cars and drivers looking for business, offering taxis in the street.

Renting a car can be a very nice solution if you want to stay in a place "far" from the centre. It is not very expensive to rent a car but, if you want to feel the city, you can make longer trips, because the city centre has a lot of small streets, that you can only enjoy walking.

WEATHER
Please visit the Portuguese Meteorology Institute website: www.ipma.pt
Or the worldwide known: www.weather.com

TOURISM AND LEISURE
The conference venue is located very near of the heart of the lively city of Braga. Just go out and enjoy! To know more about this roman origin city, please visit the following websites:
www.cm-braga.pt/pt
visitbraga.travel/
www.visitportugal.com/en/node/73738
You can also buy Time Out Porto to know what happening this month in the city.

CURRENCY
Portugal uses the Euro (€). Traveller's cheques can be exchanged for cash in banks and exchange bureaus.

EMERGENCIES
Police, ambulances, fire services: Dial 112.

LIABILITY
The Organising Committee of the conference accepts no liability for participant personal injuries or loss/damage to personal property either during or as a result of the Conference. They are entitled to make any changes, modifications or omissions with respect to the information published in this book.

INSURANCE
The Workshop Organisers cannot accept any responsibility for personal accidents and damage to the private property of Workshop and Exhibition Delegates.

SCIENTIFIC INFORMATION

ORAL PRESENTATIONS
The code attributed to the Oral Presentations in the program corresponds to the code given in this proceedings book in the abstracts list.

KEYNOTE PRESENTATION FORMAT
45 minutes presentation
15 minutes discussion

ORAL PRESENTATION FORMAT
10 minutes presentation
5 minutes discussion

CONFERENCE PROGRAM

	Day 1 | Monday |June 14	Day 2 | Tuesday |June 15	Day 3 | Wednesday| June 16
09.00 |09.30	**Registration (Foyer)**		
09.30|09.45	Opening	**KL05**	**KL09**
09.45 |10.00	Opening Lecture **KL01**		
10.00 |10.15			
10.15 |10.30			
10.30 |10.45		**OP04**	**OP08**
10.45 |11.15	*Coffee-Break*	*Coffee-Break*	*Coffee-Break*
11.15 |11.30	**KL02**	**KL06**	**KL10**
11.30 |11.45			
11.45 |12.00			
12.00 |12.15			
12.15 |12.30	**OP01**		Closing session
12.30 |14.30	*Lunch*	*Lunch*	*Lunch*
14.30 |14.45	**KL03**	**KL07**	
14.45 |15.00			
15.00 |15.15			
15.15 |15.30			
15.30 |15.45	**OP02**	**OP05**	
15.45 |16.00	**OP03**	**OP06**	
16.00 |16.15	*Coffee-Break*	*Coffee-Break*	
16.15 |16.30			
16.30 |16.45	**KL04**	**KL08**	
16.45 |17.00			
17.00|17.15			
17.15 |17.30			
17.30 |17.45		**OP07**	

** All abstract's codes are in reference to the abstracts lists published in this book.*

	Day 1 | Monday, June 14
09.00-09.30	Registration
09.30-09.45	**Welcome and Opening Ceremony** Rui L. Reis and S. C. Kundu *(3B´s Research Group, University of Minho, Portugal | FoReCaST 2021 Conference Chairs)*
	Chair: Rui L. Reis | Co-Chair: S. C. Kundu
09.45-10.45	**Opening Lecture** **KL01 - Organoids To Model Human Disease** Hans Clevers *(Hubrecht Institute and the Princess Máxima Center for Pediatric Oncology, Utrecht University, The Netherlands)*
10.45-11.15	*Coffee-Break*
11.15-12.15	**KL02 - Development of 3D tumouroids** Umber Cheema *(University of College of London (UCL), Division of Surgery & Interventional Science, United Kingdom)*
12.15-12.30	**OP01 - Collective directional migration drives the formation of heteroclonal cancer cell clusters** Miriam Palmiero *(Candiolo Cancer Institute - FPO- IRCCS, Department of Oncology - University of Turin, Italy)*
12.30-14.30	Lunch (Restaurant)
	Chair: Miguel Oliveira | Co-Chair:Catarina Abreu
14.30-15.30	**KL03 - Engineering the tumour microenvironment with biomimetic materials and systems** Subhas Kundu *(3B´s Research Group, University of Minho, Portugal)*
15.30-15.45	**OP02- Growth factors delivery system for skin regeneration: an advanced wound dressing** Marta Nardini *(Biotherapy Laboratory, Department of Internal Medicine (DIMI) University of Genova, Italy)*
15.45-16.00	**OP03- Can patient-derived organoid models guide clinical decision making in head and neck squamous cell carcinoma?** Rosemary Millen (Hubrecht Institute and Oncode Institute, Utrecht, The Netherlands)
16.00-16.30	*Coffee-Break*
16.30-17.30	**KL04- Replicating the cross-talk between cancers and bone using complex engineered models of vascularized bone tissue in-vitro, in-vivo and on-a-chip** Luiz Bertassoni *(Oregon Health and Science University (OHSU), USA)*

	Day 2 | Tuesday, June 15
	Chair: Vitor Correlo| Co-Chair: Banani Kundu
09.30-10.30	**KL05 - 2D and 3D in vitro models able to mimic the role of hyaluronic acid on gastric cancer** Ricardo Pires *(3B's Research Group, University of Minho, Portugal)*
10.30-10.45	**OP04 - Tumor-Associated Protrusion Fluctuations as a Signature of Breast Cancer Invasiveness** David Caballero *(3B´s Research Group, University of Minho, Portugal)*
10.45-11.15	*Coffee-Break*
11.15-12.30	**KL06 - Tissue repair by activation of endogenous stem / progenitor cells** Ranieri Cancedda & Milena Mastrogiacomo *(Genova University, Italy)*
12.30-14.30	Lunch (Restaurant)
	Chair: Ricardo Pires| Co-Chair: Miguel Neves
14.30-15.30	**KL07 - Bioprinting of in vitro 3D models** Miguel Oliveira *(3B's Research Group, University of Minho, Portugal)*
15.30-15.45	**OP05 - Alternative extracellular matrix formulation for growing patient-derived colorectal cancer organoids** Banani Kundu *(3B´s Research Group, University of Minho, Portugal)*
15.45-16.00	**OP06 - Establishment of Colorectal Cancer Organoids in Microfluidic-Based System** Sandra Carvalho *(3B's Research Group, University of Minho, Portugal)*
16.00-16.30	*Coffee-Break*
16.30-17.30	**KL08 - Perturbation of hyaluronan functions toward understanding and treatment of breast cancer** Iva Pashkuleva *(3B´s Research Group, University of Minho, Portugal)*
17.30-17.45	**OP07 - Carbohydrate amphiphiles as selective inhibitors of cancer cell growth** Alexandra Brito *(3B´s Research Group, University of Minho, Portugal)*

	Day 3 | Wednesday, June 16
	Chair: David Caballero | Co-Chair: Sandra Carvalho
09.30-10.30	**KL09 - Electrohydrodynamic jetting: developments, tips, and tricks in the fabrication of microfibers for tissue engineering applications** **Paul Wieringa** *(MERLN Institute, Maastricht University, The Netherlands)*
10.30-10.45	**OP08 - Modelling lung cancer metastasis through a human microcirculation-on-a-chip** **Catarina Abreu** *(3B´s Research Group, University of Minho, Portugal)*
10.45-11.15	*Coffee-Break*
11.15-12.15	**KL10 - Tumor-derived Extracellular Vesicles: From cell-cell communication to biomarkers discovery** ***Bruno Costa da Silva*** *(Champalimaud Centre for the Unknown, Lisbon, Portugal)*
12.15-12.30	**Closing session**


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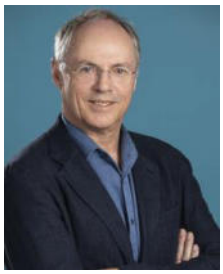
KEYNOTE LECTURES



KL01

Organoids to model human disease

Hans Clevers

Hubrecht Institute and the Princess Máxima Center for Pediatric Oncology, Professor of Molecular Genetics at Utrecht University and Oncode Investigator.
The Netherlands

Stem cells are the foundation of all mammalian life. They come in two flavors. Embryonic stem cells are briefly present in the early human or mouse embryo, a few days after fertilization. These stem cells can be grown indefinitely in the lab and have the potential to build each and every tissue in our body. ES cells hold great promise in the field of regenerative medicine.

Adult stem cells. Every organ in our body harbors its own dedicated stem cells. These adult stem cells replace tissue that is lost due to wear and tear, trauma and disease. Adult stem cells can only produce the tissue in which they reside. The adult stem cells allow us to live 80-90 years, but this comes at a cost: they easily turn into cancer.

Both types of stem cells can be used to establish 'organoids', 3D structures established in a dish, that recapitulate many aspects of the original organ -including its diseases.

Biography

Professor Hans Clevers obtained his MD degree in 1984 and his PhD degree in 1985 from the University Utrecht, the Netherlands. His postdoctoral work (1986-1989) was carried out with Cox Terhorst at the Dana-Farber Cancer Institute of the Harvard University, Boston, USA.
From 1991-2002 Hans Clevers was Professor in Immunology at the University Utrecht and, since 2002, Professor in Molecular Genetics. From 2002-2012 he was director of the Hubrecht Institute in Utrecht. From 2012-2015 he was President of the Royal Netherlands Academy of Arts and Sciences (KNAW). From 2015 - June 2019 he was Director Research of the Princess Maxima Center for pediatric oncology. He continues to run his lab in the Hubrecht Institute.
Throughout his career, he has worked on the role of Wnt signaling in stem cells and cancer. His discoveries include TCF as the nuclear Wnt effector, the role of Wnt in adult stem cell biology and of Wnt pathway mutations in colon cancer, Lgr5 as a marker of multiple novel types of adult stem cells and as receptor for the Wnt-amplifying R-spondins. Finally- a method to grow ever-expanding mini-organs ('organoids') from Lgr5 stem cells derived from a range of healthy or diseased human tissues. This has led to over 750 publications and >90,000 citations
Hans Clevers is member of the Royal Netherlands Academy of Arts and Sciences (2000), of the American Academy of Arts and Sciences (2012) and the National Academy of Sciences of the USA (2014), the Academie des Sciences (2016) and the Orden pour le Merite der Wisschschaften und Kuenste (2017).
He is the recipient of multiple awards, including the Dutch Spinoza Award in 2001, the Swiss Louis Jeantet Prize in 2004, the German Meyenburg Cancer Research Award in 2008, the German Ernst Jung-Preis für Medizin in 2011, the French Association pour la Recherche sur le Cancer (ARC) Léopold Griffuel Prize, the Heineken Prize (2012). Dr. Clevers also received the Breakthrough Prize in Life Sciences (2013), the 2015 ISSCR McEwen Award for Innovation and the Academy Professor Prize (2015), and the Körber European Science Prize (2016). He is Chevalier de la Legion d'Honneur since 2005, Knight in the Order of the Netherlands Lion since 2012 and German prize.

KL02

Development of 3D tumouroids

Umber Cheema, Marilena Loizidou, Mark Emberton, Judith Pape, Deniz Bakkalci, and Stefano Fedele

University of College of London (UCL), Division of Surgery & Interventional Science United Kingdom

In vitro models of solid tumours are being developed further to elucidate mechanisms of tumour growth, invasion and metastasis. These models are also valuable tools to measure the efficacy of specific therapeutic interventions and can be used as platforms to test novel drugs. Current model systems used include spheroid culture, matrigel cultures and organoids. We have developed 3D tumouroids as a biomimetic model with the specific aim to bioengineer the tumour and stroma component with a distinct boundary to allow for precise quantification of cancer invasion. Dependent upon the tissue of interest, we have bioengineered connective tissue stroma and bone stroma. Furthermore, we have bioengineered primitive vascular networks in the tumour stroma to enhance the biomimicry of the model. As tumouroid models are further developed to add tissue complexity and become more biomimetic, they will eventually replace early testing within *in vivo* models.

Biography

Dr. Cheema's research interests focus on 3D Bioengineering. During her PhD she engineered a 3D model of skeletal muscle to study IGF-I gene splicing in response to mechanical load. As a BBSRC David Phillips Fellow, she worked on engineering hypoxia gradients in 3D collagen scaffolds to control signalling of angiogenic genes and engineer vascular networks to understand further the role of native matrix density and composition on this biological process. She was part of the team to develop a novel plastic compression technique for collagen I hydrogels, which is now patent protected. Her research also focuses on controlling elements of collagen architecture to build biomimetic tissues in vitro. Recent research projects include developing 3D tumouroids, which are in vitro models of solid tumour growth. Here the spatial architecture of a tumour and its surrounding stroma has been reproduced in vitro, with evidence of tumour invasion into surrounding 'normal' tissue.

KL03

Engineering the tumour microenvironment with biomimetic materials and systems

Subhas C. Kundu
3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Minho, AvePark - 4805-017 Barco, Guimarães, Portugal; and ICVS/3 B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal.

The tumour microenvironment is formed by a heterogeneous collection of cells, secreted factors, and extracellular matrix proteins that influence tumour progression and therapeutic responses. Mimicking in vitro the tumour microenvironment is of utmost importance to better understand the fundamental aspects of tumorigenesis, metastasis and accurately assess cancer cells' response to novel pharmacological formulations. In this regard, engineered three-dimensional (3D) in vitro tumour models have already demonstrated their enormous capacity to recapitulate better the mechanical, (bio) chemical, structural, and cellular properties and content of the native scenario. Typically, this type of tumour models employ biomimetic materials (natural, synthetic, or blend) with advanced functional properties, which are a powerful alternative to MatrigelTM, considered the gold-standard material for tumour modelling well-known limitations. Among all the available biomaterials, silk fibroin offers a myriad of advantages for tumour modelling. For example, hydrogels and scaffolds derived from silk fibroin can be mechanically tuned (i.e.,), and their morphology precisely controlled (e.g., pore size, anisotropy). Additionally, silk fibroin is structurally homologous to collagen, the most abundant protein in the tumoral extracellular matrix. Therefore, the native content of the tumour microenvironment and in vivo-like cellular behaviours may recapitulate.

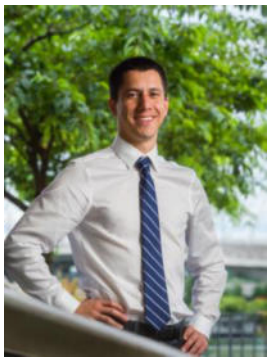
I will describe how silk biomaterial can be manipulated and engineered to develop silk fibroin-derived hydrogels and scaffolds, emphasizing their use as in vitro 3D (tumour) models. I will also introduce our current work at the 3B's Research Group – the University of Minho in the framework of the FoReCaST project, including innovative biomaterials, 3D tumour models, organ-on-a-chip systems, biophysical screening tools, and cancer organoids, and others. We anticipate that the developed biomaterials, strategies, and technologies will be valuable to cancer researchers, oncologists, and other stakeholders working in cancer modelling and drug screening.

Acknowledgements: Financially supported by European Union Framework Programme for Research and Innovation Horizon 2020 under ERA Chair grant agreement nº 668983-FoReCaST, and BREAST-IT FCT- Portugal project to SCK nº PTDC/BTM-ORG/28168/2017. I wish to record my sincere thanks to all the Academic Colleagues (present and formers) and students (former) involved in the described work, particularly the ERA Chair team members of the FoReCaST project.

Biography

Subhas C Kundu is a European Research Area Chair and Full Professor at 3B's Research Group, I3Bs - Biomaterials, Biodegradables and Biomimetics, University of Minho, Portugal. He obtained his PhD in Genetics from Banaras Hindu University, India. He received postdoctoral training at the Institute of Molecular Biology, Moscow, York University, Canada, Medical University, Lubeck, Germany, and Brunel University, United Kingdom. He was the Founder, Head, and Full Professor of the Department of Biotechnology, Indian Institute of Technology Kharagpur (IIT), India, and Distinguished Invited Professor, Dankook University, South Korea. In his early teaching career, he was Assistant to Full Professor and Head of Department of Life Sciences, Manipur University, Imphal. He taught genetics, cell and molecular biology, and recombinant DNA technology to under-graduates, post-graduates and pre-doctoral students. His current research interest is on Natural biomaterials, drug delivery systems, 3D cancer modelling and drug screening. He has published 190 major research articles (H-index 62, and citations 17,781) in peer-reviewed high impact factor journals like Progress in Polymer Science, Biotechnology Advances, Advanced Drug Delivery Reviews, Acta Biomaterialia, Biomaterials, Journal of Control Release,

Biomacromolecules, ACS Applied Materials and Interfaces, Advanced Functional Materials, Advanced Materials, and others.



K04

Replicating the cross-talk between cancers and bone using complex engineered models of vascularized bone tissue in-vitro, in-vivo and on-a-chip

Luiz Bertassoni

Oregon Health and Science University (OHSU), Portland, Oregon, USA.

Bone tissue, by definition, is an organic-inorganic nanocomposite, where metabolically active cells are embedded within a matrix material that is heavily calcified on the nanoscale. Replicating these critical hallmarks of bone tissue has remained highly elusive for many years. Our group has developed a series of biomimetic approaches where polymeric agents are used as protein analogues with supersaturated calcium and phosphate solutions to direct the nanoscale deposition of hydroxyapatite in the interstices of collagen nanofibrils embedded with cells. Using this process, we have engineered bone models replicating the unique hallmarks of the bone cellular and extracellular microenvironment, including its method of biomineralization, nanostructure, mechanics, vasculature, innervation, and inherent osteoinductive properties. Currently, this innovative technology is being used as a unique model to study cancer metastasis into bone. Bone is the most common site of metastasis for several types of cancers, including breast, lung, and prostate, which metastasizes into the bone in ~90% of patients. The mechanisms regulating such trophic effect remain largely unknown. Here we demonstrate that such engineered bone tissue can attract prostate cancer in-vitro and in-vivo. We then miniaturize these engineered models to generate the first organ-on-a-chip model system replicating the dislodgement of prostate cancer from a tumor mass, its intravasation into a mature vasculature, and extravasation into a bone-like nano-mineralized osteoblast-rich matrix. In this talk, we propose the hypothesis that the stiff bone matrix contributes to the dystrophic modifications of the prostate cancer cell nucleus, which appears to contribute to the tumorigenic potential and aggressiveness of metastatic prostate cancer cells.

Relevant references:

- Rapid fabrication of vascularized and innervated cell-laden bone models with biomimetic intrafibrillar collagen mineralization. Nature Communications volume 10, Article number: 3520 (2019)
- Bone-on-a-Chip: Micro uidic Technologies and Microphysiologic Models of Bone Tissue. Advanced Functional Materials. 2020, 2006796.

Biography

Dr. Luiz Bertassoni is an Associate Professor at Oregon Health and Science University (OHSU). He holds joint appointments in the School of Dentistry, the Department of Biomedical Engineering and the Cancer Early Detection Advanced Research center (CEDAR) at the Knight Cancer Institute. He concluded a PhD in Biomaterials at the University of Sydney and two postdoctoral fellowships at the University of California San Francisco and the Harvard-MIT's Division of Health Sciences and Technology. Dr. Bertassoni's work encompasses various aspects of micro-scale technologies and bioprinting for tissue regeneration; nanoscale properties and fabrication of vascularized and mineralized tissues; and different aspects in the field of organs-on-a-chip. His research has consistently appeared in journals such as Nature Communications, Advanced Materials, Advanced Functional Materials, Biofabrication, and others. He has published over 70 manuscripts, received over 30 international

research awards, is a co-founder of two start-up companies, and serves as an associate editor for three international journals.

KL05

2D and 3D in vitro models able to mimic the role of hyaluronic acid on gastric cancer

S. Amorim[1,2], D. Soares da Costa[1,2], S. Mereiter[3,4], I. Pashkuleva[1,2], C. A. Reis [3,4,5,6], R. L. Reis[1,2], R. A. Pires[1,2]

[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Guimarães, Portugal
[2]ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal
[3]Instituto de Investigação e Inovação em Saúde - i3S, Universidade do Porto, Portugal
[4]Institute of Molecular Pathology and Immunology of the University of Porto – IPATIMUP, Portugal
[5]Department of Pathology and Oncology, Faculty of Medicine, Porto University, Porto, Portugal
[6]Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal

Gastric cancer (GC) is one of the most common types of cancers. During its onset, GC is confined to a primary site that progresses to later cancer stages, initiates the process of cancer cell invasion, and the formation of metastasis to secondary sites reduces. This metastisization process reduces the efficiency of the current treatment strategies due to the spreading of the cancer cells to different tissues. There is a clear link between the invasive character of GC cells and the composition and properties of the tumor microenvironment (TME), namely the concentration and molecular weight (Mw) of hyaluronic acid (HA). In this context, there is a growing need to develop model systems able to recapitulate the TME (and its ability to modulate cancer cell behaviour) as platforms to study cancer-related biochemical cascades and evaluate the efficacy of drugs. We developed a series of 2D and 3D models, that mimic the physiological presentation of HA on the extracellular matrix (ECM). We studied the influence of its Mw on the adhesion, migration and invasiveness of GC cells. 2D surfaces generated by Layer-by-Layer assembly can present HA in an ECM-relevant manner, being recognized by its specific cell surface receptor, CD44. HA of low Mw (6.4kDa) promoted the migration of i) AGS cells, in a process mediated by RHAMM and through the activation of the AKT signaling cascade; and ii) MKN45 cells, through a paxillin-mediated activation of focal adhesion sites. We also developed an Alginate-HA 3D model, including the co-culturing MKN45 (GC cells) and healthy mesenchymal stem cells (MSCs). In this case, we designed the system to present a core of MKN45 cells (mimicking the primary cancer site) and a shell of healthy MSCs. We demonstrated that this system is a faithful minimalistic mimic of the TME: the presence of HA of high Mw (i.e., 1500kDa) promoted the migration of MSCs into the MKN45 cancer cell niche; HA of low Mw induced the migration of the MKN45 cells into the shell of the 3D model. In summary, we show that it is possible to mimic the biological role of HA using the developed models and that GC cells respond differently to the Mw of HA.

Acknowledgements: This work was financially supported by the EC, under grant agreement 668983–FORECAST.

Biography

Ricardo A. Pires graduated in Chemistry from the University of Lisbon (1998) and has a PhD in Materials Engineering from the Technical University of Lisbon (2004). He has worked in Corticeira Amorim SGPS (2005-2010) to use natural-based compounds extracted from cork as anti-oxidant and anti-inflammatory agents. Since 2010, he is an Assistant Researcher at the University of Minho (3B's Research Group), working in biomaterials, tissue engineering, and regenerative medicine. His main research interests are related to the development of 2D and 3D cancer models; the modulation of protein aggregation driven by self-assembly, e.g. amyloids; the design of supramolecular hydrogels generated by the self-assembling of short peptide and carbohydrate

amphiphiles that can mimic the morphological and biofunctional characteristics of the proteins and proteoglycans present in the extracellular matrix; the exploitation of nanotechnological tools (e.g., lithography, etc.) in the biomedical area; as well as the development and use of organic and inorganic bionanomaterials, including modified silica-based nanoparticles for the stimulation of stem cell differentiation. During his research career, he authored 58 papers in an international scientific journal with refereeing (h-index of 17), 4 patents, 6 conference proceedings, 5 book chapters and approx. 100 conference communications. He has published his work in high-impact international journals, such as Journal of the American Chemical Society, Chemical Science, Chem, Progress in Polymer Science, Materials Today, ACS Applied Materials and Interfaces, Acta Biomaterialia, and others.


KL06

Tissue repair by activation of endogenous stem / progenitor cells

Ranieri Cancedda & Maddalena Mastrogiacomo

Universita' di Genova, Italy

Cell therapy approaches, i.e. transplantation of "ex vivo" expanded autologous stem/progenitor cells, alone or associated to carrier biomaterials can be life/organ saving and allow treatments of very critical patients. However, due to difficult logistics, regulatory issues requiring the adoption of highly sophisticated cell culture facilities, and the high cost of the procedures, these approaches cannot be applied for largely diffuse, difficult to heal tissue deficits such as chronic skin ulcers or osteoarthritis. To enable a large number of patients to benefit from a Regenerative Medicine approach, new strategies and new products should be considered. The use of media conditioned by progenitor / stem cells or of extracellular vesicles and exosomes released by the same cells is being presently investigated. Alternatively, the treatment of the tissue deficits with platelet derived components has been proposed. In almost all injured tissues, there is a common initial stage after the damage, characterized by hematoma, clot formation and platelet activation. Activated platelets release a variety of growth factors and bioactive molecules that play a significant role in triggering the healing process and are crucial for regulating immune cell migration and the creation of an inflammatory microenvironment at the site of the wound. Following this first inflammatory phase, repair or regeneration of the tissue occur through paracrine signals activating the revascularization of the wound and recruiting or turning on at the wound site cells with healing potential, such as stem cells, progenitors or undifferentiated cells derived from tissue differentiated cells

Biography

Ranieri Cancedda MD, Professor Emeritus of Cell Biology and past Dean of the Biotechnology School at the University of Genova, Italy, also served as Coordinator of the PhD program in Biotechnology in Translational Medicine in the same university and as Head of the Laboratory of Regenerative Medicine at the teaching hospital San Martino-IST, Genova.
One of the founders and first President of the European Tissue Engineering Society, now the European Chapter of Tissue Engineering and Regenerative Medicine International Society (TERMIS), is currently a "Affiliate Member" of the Mc Gowan Institute for Regenerative Medicine, University of Pittsburgh, USA e "Fellow" of TERMIS. He is the author of more than 350 publications (h-index 86; citations >30,000), and inventor in 9 patents. Dr. Cancedda is Field Chief Editor of Frontiers in Bioengineering and Biotechnology and Specialty Chief Editor of the Regenerative Medicine and Tissue Engineering section.

Dr. Maddalena Mastrogiacomo has a degree in Biological Sciences in 1996 from the University of Bari and doctoral in Cellular Biology in 2002 from the University of Torino. Her main research field include cell biology of mesenchymal progenitor cells, tissue engineering using biomaterials. She developed her expertise in the Laboratory of Regenerative Medicine at the National Cancer Research, Genova, Italy. focusing her work on pre-clinal and clinical study to bone tissue regeneration. She is currently employed at the Laboratory of Bioteraphy of the University of Genoa as researcher. Dr. Mastrogiacomo is author of 80 publications in scientific journals and has been selected to present her works in national and international congresses. For many years she has been working on the use of Mesenchymal Stem Cells in bone regenerative medicine and more recently he has been working on the efficiency of platelet derivatives in the repair and healing of skin tissue. In this direction she is responsible for a clinical trial conducted at the San Martino Polyclinic Hospital in Genoa which provides for the treatment of severe ulcers in diabetic patients using platelet gel.
She is responsible of the training program of graduated students in Biotechnology and Regenerative Medicine. She is professor at the Faculty of Medicine, Biotechnology and Healthy Professions at the University of Genoa. The research group coordinated by her is focused on the skin and bone tissue regeneration.

KL07

Bioprinting of in vitro 3D models

Joaquim Miguel Oliveira

3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Minho, AvePark - 4805-017 Barco, Guimarães, Portugal; and ICVS/3 B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal.

Tissue engineering principles and products have been proposed for different applications, as they can be applied to test well-known and new anti-cancer drugs, as drug delivery systems of chemotherapeutic agents, or even as in vitro 3D models mimicking tissue/organ microenvironment and complexity, the so-called complex in vitro models (CIVMs). By its turn, emerging technologies such as 3D bioprinting, an extension of additive manufacturing technique, has introduced a new dimension to 3D in vitro models by means of allowing the generation of a highly controlled CIVMs. The bioprinting technique has been especially relevant in the cancer field since it allows to engineer the complex microenvironment that cancer cells are subjected to, disclosing important insights concerning cell-cell and cell-ECM interaction. In order to create proper 3D bioprinted CIVMs of the disease and tumours, it is crucial to select the most suitable biomaterial with the capability to be printable and allow the inclusion of relevant cells, as known as, bioink, and the most suitable printing methods. The recent reports and research contributions developed at 3B's dealing with different bioinks used in bioprinting of in vitro 3D models will be discussed.

Biography

J. Miguel Oliveira BSc, Ph.D. (Portuguese, M, 43 years old) is a Principal Investigator at the 3B's Research Group, Institute 3Bs, University of Minho. He is also the Vice President of I3Bs - Institute 3B's (Univ. Minho) and Director of Pre-Clinical Research at the FIFA MEDICAL CENTER, Estádio do Dragão, Porto, PT since Feb. 2013. Currently, he is a Lecturer in Doctoral Program in Tissue Engineering, Regenerative Medicine and Stem Cells (TERM&SC) at UMinho, PT (since Dec. 2013). He was also an invited lecturer at the Faculty of Medicine, U. Porto (2013-2019) and Dept. of Polymer Eng., UM, PT (2009-present). Over the years he has focused his research work on the field of biomaterials for tissue engineering, nanomedicine, stem cells, and cell/drug delivery. More recently, he set up a new research line within the ICVS/3B's on 3D in vitro models for cancer research. As a result of his proficiency, he has published so far more than 300 scientific contributions in scientific journals with the referee, being 4 of those review papers produced under invitation. Miguel Oliveira has approved more than 20 patents (filled & submitted), published 6 books (plus 2 in preparation), 5 special issues in scientific journals, and more than 90 book chapters in books with international circulation. He has participated in more than 500 communications in national/international conferences and has been invited/keynote speaker in more than 50 plenary sessions. He has an h-index of 49, i10 of 156 and received more than 9000 citations. He has been awarded several prizes including the prestigious Jean Leray Award 2015 from European Society for Biomaterials for Young Scientists for Outstanding Contributions within the field of Biomaterials. He is very active on the elaboration and scientific coordination of several PT and international funded projects. In addition, he is member of the National Ethics Committee for Clinical Trials from Serviço Nacional de Saúde (SNS) (PORTUGAL). He is the Editor-in-chief of the In vitro Models Journal (Springer Nature). He also integrates the advisory /editorial board of the Journal Bio-Design and Manufacturing, Journal of Materials Science: Materials in Medicine, Materials (MDPI), International Journal of Tissue Engineering, Journal of Composites and Biodegradable Polymers, Journal ISRN Biomaterials, The Journal of Experimental Orthopaedics, Journal "Recent Patents on Corrosion Science", and referee in more than 50 international journals. In 2020, he was listed among the TOP50 worldwide experts in tissue engineering by EXPERTSCAPE (https://expertscape.com/ex/tissue+engineering).

KL08

Perturbation of hyaluronan functions toward understanding and treatment of breast cancer

Iva Pashkuleva[1,2]

[1]3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Guimarães, Portugal
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Hyaluronan (HA) is a non-sulfated glycosaminoglycan with critical roles in various physiological processes. Its amount is tightly controlled and regulated by several synthetases and hyaluronidases but during carcinogenesis, HA homeostasis is hijacked by cancer cells and the altered synthesis and degradation processes result in specific HA turnover profile within the tumor microenvironment.1,2 HA concentrations are usually higher in malignant tumors than in corresponding benign or normal tissues, and in patients with breast carcinomas, the increased HA concentration in the stroma is associated with low survival rates.2 Besides these clinical evidences, the signaling pathways leading to this negative outcome are yet to be elucidated. Herein, we present several methodologies that were developed by us recently and applied toward understanding of the crosstalk between HA and its main receptors - CD44 and RHAMM - in breast cancer. Keeping in mind the physiological presentation of HA, we established methods for its immobilization that preserves its bioactivity and demonstrated that immobilized HA induces amplification of signal transduction as compared with its soluble analogue.1,3,4 This amplification depends on HA density and the expression level of CD44 and RHAMM. We also evidenced the compensatory effect between CD44 and RHAMM in the signal transduction that is consistent with the concept of the molecular redundancy.1 Apart from these mechanistic findings, we have developed several nanotherapeutics that target HA-CD44 pathway and are based on end-on immobilized low molecular weight HA. These include stimuli-responsive nanoparticles that can encapsulate either hydrophilic or hydrophobic drugs and deliver these via CD44 selective targeting3 and brush-like copolymers5 that block the CD44 clustering and signal transduction.
Acknowledgments: European Union H2020 program (H2020-WIDESPREAD-2014-2-668983-FORECAST) and Portuguese Foundation for Science and Technology (CardioHeal: PTDC/BTM-MAT/28327/2017, CANCER_CAGE PTDC/NAN-MAT/28468/2017, CARTI_LIKE PTDC/QUI-POL/28117/2017).

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Biography

Iva Pashkuleva gained her PhD degree in Organic Synthesis at the University of Sofia, Bulgaria. After finishing her degree, she worked as an Assistant Professor of Organic Chemistry at the same University for two years and then she moved to 3B's Research group, University of Minho, Portugal, where she started research activities within the field of carbohydrates based biomaterials. In 2008, she was awarded Career Starting Grant under the National Program Compromisso com a Ciência. During this period, her research was focussed on surface modification of biomaterials (with emphasis on starch and chitosan) as a way to enhance their biocompatibility. In 2013, she was awarded a Development Career Grant under the Program Investigador FCT to work on the elucidation of glycan role in cellular communication and the possibility to explore glycans in design and synthesis of functional biomaterials. Currently, Iva Pashkuleva is a Principal Investigator at the 3B's Research group and her research activities are focused on the development of new analytical methods and platforms for characterization of challenging to measure glycan-protein and glycan-cell interactions, design and synthesis of glycan-based delivery systems and extracellular mimics, and self-assembly of glycan amphiphiles toward biofunctional materials.

KL09

Electrohydrodynamic jetting: developments, tips, and tricks in the fabrication of microfibers for tissue engineering applications

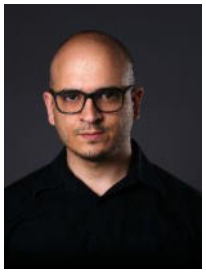
Paul Wieringa

Complex Tissue Regeneration Department, MERLN Institute, Maastricht University, The Netherlands

In the pursuit of creating more effective scaffolds for regenerative medicine and tissue engineering applications, electrohydrodynamic (EHD) jetting has found extensive use as an approach to generate polymeric fibers reminiscent of the extracellular matrix. Electrospinning, in particular, is an EHD technique applied to polymer solutions that have been widely shown to produce nano- to micron-sized fibers capable of effectively mimicking the fibrous extracellular matrix structure and influencing cell growth. However, this fabrication approach is intrinsically chaotic due to the physical principles involved, placing limitations on scaffold production and requiring specialized techniques to create tailor-made scaffold designs. More recently, the development of melt electrowriting technologies have provided a more controlled approach for generating microfiber scaffolds via the deliberate collection of EHD jetted microfibers generated from a molten polymer melt. Again, however, this approach also comes with seemingly intrinsic limitations while giving new possibilities for creating tissue-engineered scaffolds. Presented here is an exposé of ongoing developments in electrospinning and melt electrowriting technologies, providing insight into fabrication mechanisms, strategies to overcome typical challenges to scaffold manufacturing, and showcasing applications of the resulting fibrous scaffold designs.

Biography

Dr. Paul Wieringa received his M.Sc. (cum laude) in Electrical Engineering from the University of Twente in 2009. He completed a co-Ph.D. in 2014 from the University of Twente and Scuola Superiore Sant'Anna, Italy (cum laude) on the development of 3D electrospun scaffolds for nerve regeneration. From 2014 to 2017, Paul held a postdoctoral fellowship within the MERLN Institute. He focused on the technological development of electrospun tissue scaffolds and their application in nerve regeneration and regenerative medicine. In 2017, he was awarded the prestigious VENI personal grant and was promoted to Assistant Professor in 2018. His recent activities include expanding traditional solution-based electrospinning and the development of melt electrospinning writing approaches. Through applying these technologies, his biological focus is the realization of 3D in vitro platforms to study and model the innervation of tissues and explore the neural influence on tissue pathologies and tissue repair mechanisms.



K10

Tumor-derived Extracellular Vesicles: From cell-cell communication to biomarkers discovery

Bruno Costa da Silva
Systems Oncology Group, Champalimaud Research, Champalimaud Centre for the Unknown, Lisbon, Portugal

Last 15 years, research on the metastatic progression of cancer has shown that tumors can modify normal tissues at a distance by releasing extracellular vesicles. They travel in the bloodstream, bind to distant cells and transfer oncoproteins that ultimately promote the formation of microenvironments prone to receive and support metastatic lesions, formed even before the arrival of the first metastatic cells, known as pre-metastatic niches. We discovered that pancreatic cancer-derived exosomes are carrying high levels of macrophage migration inhibitory factor (MIF) bind preferentially to Kupffer cells in the liver, inducing production of inflammatory mediators such as TGF-B. This promotes in turn, extracellular matrix remodelling by hepatic stellate cells that support the accumulation of bone marrow-derived macrophages, which ultimately contribute to the attachment and growth of metastatic pancreatic cancer cells in the liver. Compared with patients whose pancreatic tumors did not progress, MIF was markedly higher in exosomes from stage I PDAC patients who later developed liver metastasis, suggesting that exosomal MIF may be a prognostic marker for the development of PDAC liver metastasis. Furthermore, we also showed that exosomal patterns of integrins expression dictates the tissue affinity of tumor exosomes, which in turn determines the location of pre-metastatic niches formation and the tumor metastasis organ distribution. Our clinical data indicate that exosomal integrins could be used to predict organ-specific metastasis, helping to answer one of the greatest mysteries of metastatic cancer regarding the biological basis of organotropism.

Biography

During my PhD, I studied how the interaction between Prion Protein and its ligand HSP70/HSP90 Organizing Protein induces colorectal cancer cells migration and invasion, glioblastoma growth and exosome production. I played a vital role in the first publication to show how tumor exosomes induce the formation of pre-metastatic niches. During my post-doc, I authored the first work to identify the hepatic pre-metastatic niches' mechanism by pancreatic cancer exosomes. I also participated in the first description of genomic double-stranded DNA packaging in tumour-derived exosomes. I co-first authored a work that introduced the molecular basis of how tumor-derived exosomes form pre-metastatic niches in specific organs. As a group leader at Champalimaud Foundation, I maintain productive collaborations with our Clinical Center and other European institutions. My group focuses on identifying the roles of cancer EVs and identifying exosomes populations linked to the incidence and prognosis of oncologic diseases.

ORAL LECTURES

OP1

Collective directional migration drives the formation of heteroclonal cancer cell clusters

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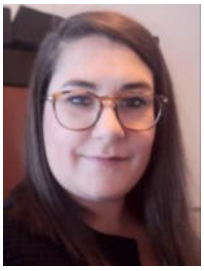
[2]2Univeristy of Turin- San Luigi Hospital, Department of Clinical and Biological Sciences, Orbassano, Italy.

Metastatization occurs through the acquisition of invasive and survival capabilities that allow tumour cells to colonize distant sites. While the role of multicellular aggregates in cancer dissemination is acknowledged, the mechanisms that drive the formation of multi-clonal cell aggregates are not fully elucidated. Here we show that cancer cells of different origins can perform collective directional migration and actively form heteroclonal aggregates in 3D. Coalescence of distant cell clusters is mediated by subcellular actin-rich protrusions, and multicellular outgrowths extend towards neighbouring aggregates. Coherently, perturbation of cytoskeletal dynamics impairs collective migration while myosin II activation is necessary for multicellular movements. We put forward the hypothesis that cluster attraction is mediated by secreted soluble factors consistently with the abrogation of aggregation by inhibition of PI3K/AKT/mTOR and MEK/ERK. This indicates that the conditioned culture media act as a chemoattractant. Our results present a novel collective migration model and shed light on the mechanisms of the formation of heteroclonal aggregates in cancer.

Biography

Miriam Palmiero was born in Rome (Italy) in 1988. She obtained my Bachelor's degree in Biological and Molecular Science and my Master degree in Molecular and Industrial Biotechnology at the University of Pisa. She spent one year at the University College of London to prepare my Master thesis to investigate the differentiation potential of pediatric adipose-tissue derived stem cells. Then she moved to Turin to start a PhD in Complex Systems for Life Sciences, and she is currently finishing this path at the Candiolo Cancer Institute FPO. During the last four years, she studied the collective aggregation dynamics of cancer cells in three-dimensional culture systems. She acquired experience in imaging biological processes with particular reference to cancer cell migration and expertise in long time-lapse experiments (weeks) to investigate tumour cell and organoid dynamics.



OP2

Growth factors delivery system for skin regeneration: an advanced wound dressing

Marta Nardini*, Sara Perteghella, Luca Mastracci, Federica Grillo, Giorgio Marrubini, Elia Bari, Matteo Formica, Chiara Gentili, Ranieri Cancedda, Maria Luisa Torre and Maddalena Mastrogiacomo
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Standard treatments of chronic skin ulcers based on the direct application of dressings still present several limits concerning complete tissue regeneration. Innovative strategies in tissue engineering offer materials that can tune cell behavior and promote growth tissue favoring cell recruitment in the early healing stages. A combination of Alginate, Sericin, with Platelet Lysate, as a freeze-dried sponge, is proposed to generate a bioactive device to treat skin defects. Biomembranes at different compositions were tested to release platelet growth factors, cytotoxicity, and protective effects against oxidative stress and cell proliferation induction. The highest level of the growth factors release occurred within 48 hours, an optimized time to burst a healing process in vivo; SS differently modulated the release of the factors by interaction with the proteins composing the biomembranes. The sponges did not present any cytotoxicity, whereas a capability to protect cells against oxidative stresses was observed when membranes included the PL, inducing cell proliferation. In a mouse skin lesion model, both control (empty membrane) and PL loaded membranes formed a gel absorbing the exudate produced during the wound healing process. A non-complete healing resolution was observed in the control lesions, while the presence of PL induced an accelerated and more pronounced burst of inflammation, formation of granulation tissue and new collagen deposition, leading to more rapid skin regeneration.

Biography

Dr. Marta Nardini graduated in Molecular and Industrial Biotechnology at the University of Pisa (IT) in 2013. She obtained her PhD in Translational Medicine at the University of Genova (IT) in 2018, attending the Laboratory of Regenerative Medicine managed by Prof. Ranieri Cancedda. Her main research field, which is included in regenerative medicine, focuses on the study of Mesenchymal Stem Cells derived from different sources and platelet growth derivatives to their potential application in human therapy. She was involved in several publications in the scientific journal regarding biomaterials and platelet derivatives for skin regeneration during her career. She is currently employed at the Laboratory of Biotherapy at the University of Genova as Post Doc under the scientific coordination of Dr. Milena Mastrogiacomo. Marta is continuing her previous works, and she starts to develop a new tumor model in a 3dimensional system for studying the tumor environment and immune system.

OP3

Can patient-derived organoid models guide clinical decision making in head and neck squamous cell carcinoma?

Rosemary Millen[1], Else Driehuis[1,2], Willem de Kort[2], Mandy Koomen[1], Remco de Bree[3], Lot Devriese[4], Stefan Willems[5], Robert van Es[3], Maurice Zandvliet[7] and Hans Clevers[1,6]

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Head and neck squamous cell carcinoma (HNSCC) is the 7th most common cancer worldwide [1]. More than 60% of patients present with late-stage disease and have a high risk of recurrence (15-40%) within the first two years after therapy [2]. Treatment may involve surgery and/or radiotherapy or chemoradiotherapy. Methods to predict which patients will benefit from these therapies and develop novel therapies are urgently needed and may assist clinicians in determining more personalized treatment plans. Patient-derived organoid models allow for long-term expansion and maintenance of primary epithelial cells in 3D [3]. Organoids can be established within 7-10 days of biopsy, expanded long-term, and closely recapitulate tumor heterogeneity in vitro [4].
In our observational clinical trial, we aim to evaluate the potential of organoids for personalized medicine. In vitro organoid drug response will be correlated to the clinical response of HNSCC patients. This study is unique, as we focus on primary, untreated, non-metastasized cancer. We have biobank organoids from 70 patients with HNSCC of various anatomical locations. We have undertaken in vitro radiotherapy and chemoradiotherapy drug screening in 22 organoid lines, of which 12 can be correlated with clinical response. Four patients (33%) relapsed within 12 months, and 3 of these corresponding organoids showed a more resistant phenotype in vitro when assessing AUC. In addition, we have characterised the organoid lines at the genomic level and perform targeted therapy drug screens. Patient recruitment and drug screening are ongoing, and we believe this will provide insight into patient response and new therapeutics for HNSCC patients.

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Biography

Rosemary Millen completed her PhD in 2019 at the University of Melbourne, Australia. Her research focused on understanding the function of tumor-infiltrating lymphocytes (TILS) in colorectal tumors. To investigate this, Rosie and colleagues developed an in vitro co-culture system using patient-derived organoids and autologous TILs. Rosie moved to The Netherlands in September 2019 to undertake her postdoc at the Hubrecht Institute in the lab of Professor Hans Clevers. She is now working on a clinical trial establishing organoids from patients with head and neck cancer. This project will focus on performing drug screens in vitro and correlating this with clinical response. She will also continue to investigate TIL function using organoid-TIL co-cultures.

OP4

Tumor-Associated Protrusion Fluctuations as a Signature of Breast Cancer Invasiveness

David Caballero*, Virginia Brancato, Ana C. Lima, Catarina M. Abreu, Nuno M. Neves, Vitor M. Correlo, Joaquim M. Oliveira, Rui L. Reis, Subhas C. Kundu

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ICVS/3B's – PT Government Associate Laboratory, 4805-017, Braga/Guimarães, Portugal.

During dissemination, metastatic (individual) cancer cells can modulate the morphodynamics of invasive protrusions to optimize their migration efficiency. However, it remains unclear how the stochastic fluctuations of protrusions regulate the invasion of more complex multi-cellular tumors and how they correlate with their metastatic potential. Herein, we used a reductionist approach based on tumor cell micro-spheroids with increasing invasion capability to investigate the role of fluctuating protrusions in breast cancer progression. To quantitate protrusion fluctuations, we defined a set of key biophysical parameters that precisely correlated with the invasive potential of tumors. We showed that by perturbing protrusion activity using standard chemotherapeutics and pharmacological inhibitors of key signaling pathways, tumor invasiveness was significantly altered. Finally, we defined a novel quantitative index encoding a minimal set of biophysical parameters and the relative levels of cell-cell/ECM interactions, which was capable of assessing tumor invasion capability. Overall, this work provides a new biophysical framework showing how protrusion fluctuations regulate tumor cell invasion, suggesting that they may be employed as an early indicator – signature – of the metastatic potential of tumors.

Acknowledgements: D.C. acknowledges the financial support from the Portuguese Foundation for Science and Technology (FCT) under the program CEEC Individual 2017 (CEECIND/00352/2017). D.C., A.C.L., C.M.A., and S.C.K. also acknowledge the support from FCT under the 2MATCH project (02/ SAICT/2017—028070) funded by the Programa Operacional Regional do Norte supported by FEDER. Finally; all the authors acknowledge the financial support from the EU Framework Programme for Research and Innovation Horizon 2020 on Forefront Research in 3D Disease Cancer Models as in vitro Screening Technologies (FoReCaST—no. 668983).

Biography

David Caballero is a biophysicist with an MSc and PhD in Nanoscience from the University of Barcelona (Spain). In 2009, David moved to the prestigious ISIS and IGBMC Institutes at the University of Strasbourg (France) as a postdoctoral researcher to work in cell physics. In 2014, Dr. Caballero moved to IBEC in Barcelona as a Marie Curie fellow to start a new research line on innovative 3D tumor models. His research focused on the biomechanical regulation of cancer cell invasion, particularly in the crosstalk between cancer cells and the surrounding stroma. In 2017, Dr. Caballero moved to the 3B´s Research Group at the University of Minho (Portugal) as an Assistant Researcher. His main research interests focus on developing novel tumor-on-a-chip devices and in the biophysics of cancer.

OP5

Alternative extracellular matrix formulation for growing patient-derived colorectal cancer organoids

Kundu B[1,2], Vara Messler M[3,4], Brancato V[1,2], Oliveira JM[1,2], Correlo VM[1,2], Reis RL[1,2], Primo L[3,4], Kundu SC[1,2]

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[2] ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal
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Patient-derived organoids (PDOs) hold great potential in cell therapy, developmental biology, disease modeling and drug screening. However, the translation of organoid technology into regenerative medicine is limited due to the lack of a suitable and reproducible extracellular matrix. Currently, mouse basement membrane extract (BME) hydrogel is the popular choice for in vitro culture. Hence, there is considerable interest in a defined synthetic or natural extracellular matrix that can support PDO growth. Herein, we propose gellan gum - silk fibroin blended porous sponges as an alternative to BMEs hydrogel for in vitro culture of colorectal cancer (CRC)-PDOs. We have created a biomaterial library of porous sponges using silk fibroin (SF), gellan gum (GG) - bacterial exopolysaccharide mimicking ECM glycosaminoglycan, and blending silk fibroin to gellan gum in a ratio of 1 to 1 (GG: SF). We have used a panel of CRC-PDOs to investigate the potentiality of these biomaterials to grow them in vitro. Moreover, we incorporated soluble BME (2% v/v) as a culture media supplement to partially mimic the adhesive properties of collagen-type IV and laminin. The results indicated that soluble BME facilitated the development of CRC-PDOs, inducing the formation of lumen-like structures in all sponges, greater in blended sponges. However, the cell sources play an essential role in the formation of lumen-like structures. Hence, the combination of GG: SF sponges with soluble BME can be considered as a promising matrix ECM formulation alternative to BME hydrogel for in vitro organoid culture.

Acknowledgements
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Biography

Banani Kundu obtained her Ph. D from IIT Kharagpur, India, in biomaterial designing and regenerative medicine. She was a postdoctoral research fellow at Dankook University, South Korea. Currently, she is an Assistant Researcher in 3B's Research Group, University of Minho, Portugal. Her research vision is to explore the diverse potential of proteins, ranging from designing vaccine (by identifying the smallest digested peptide fragment that possesses antigenicity, resulting in a reduction in injecting peptide size into animals during vaccine production) to diagnostic biomaterial tools to disease modeling. Her current research interest is in mechanically tunable biomaterials that are designed to unwind the regulatory effect of physical cues in cancer cell invasion and metastasis to identify new therapeutic targets of cancer. She also serves as an Editorial Board Member in Frontiers in Bioengineering (Review Editor), Biomedical Research & Experimental Sciences and Journal of Functional Materials and Chemical Engineering.

OP06

Establishment of Colorectal Cancer Organoids in Microfluidic-Based System.

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Cancer is a major leading cause of death worldwide and a target of basic and translational research. Microfluidic cell culture technology has emerged as a promising tool in cancer research. However, tumour-on-chip relies on pre-differentiated cells, often cell lines, and cannot emulate the histological and cellular complexity of the tumour, and the surrounding microenvironment. Patient-derived tumour organoids are considered preclinical models with the potential for preclinical drug screening, prediction of patient outcomes, and guiding optimized therapy strategies at an individual level. Combining microfluidic technology with 3D tumour organoid models to recapitulate tumour organization and in vivo functions led to the development of an appropriate preclinical tumour model, organoid-on-a-chip, paving the way for personalized cancer medicine. Herein, a low-cost microfluidic device suitable for culturing and expanding organoids was developed. Patient-derived colorectal cancer organoids were cultured within a microfluidic device, and their viability and proliferative activity increased significantly. No significant differences were verified in the organoids' response to 5-fluorouracil (5-FU) treatment on-chip and on-plate. However, the culture within the microfluidic device led to a significant increase in colorectal cancer organoid-forming efficiency and overall size compared with conventional culture on a plate. Interestingly, early-stage and late-stage organoids were predominantly observed on-plate and within the microfluidic device, respectively. The patient-derived colorectal cancer organoid-on-chip developed in this study has thus the potential to generate in vivo-like organotypic structures for disease modelling and drug screening applications.

Biography

Sandra Carvalho (S.C) developed her PhD work in glycobiology in the cancer field at IPATIMUP-University of Porto from 2012-2015. In 2017, S.C started postdoctoral activities in the International Iberian Nanotechnology Laboratory. Her interests included quantitative phenotyping and molecular characterization of circulating tumour cells and other rare cells isolated by microfluidic-based systems fabricated in-house for diagnosis and real-time monitoring of cancer. In parallel, S.C was involved in an exploratory research project in collaboration with the industrial partner Cellesce, Ltd from 2019 to 2020. This project aimed to develop a patient-derived organoid on-chip as a new preclinical cancer model. In February 2021, S.C was integrated into the international funded project FoReCaST at 3B's Research Group, Institute on Biomaterials, Biodegradables and Biomimetics of the University of Minho, Avepark, Guimarães, Portugal. S.C is interested in boosting her scientific path by combining the innovative microfluidics and organoids approaches and oncology knowledge to create a next-generation patient-derived 3D cancer model with the potential for personalized medicine.

OP07

Carbohydrate amphiphiles as selective inhibitors of cancer cell growth

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Biocatalytic self-assembly (BSA) consolidates the selectivity of an enzymatic conversion with the sensitivity of the self-assembly process. This approach can be applied toward cancer treatment and proved to be efficient in two-dimensional (2D) flat cell cultures.1 Such cultures, however, do not recapitulate the complexity and heterogeneity of the tumor microenvironment. Thus, more complex 3D models, e.g. spheroids, are needed to validate the approach prior in vivo tests.2 Spheroids recapitulate most important tumour characteristics such as cells heterogeneity, nutrients and metabolites diffusion, extracellular matrix remodelling and altered signalling pathways. These characteristics are particularly important for BSA application, where proteins with altered expression are used as target and/or trigger. Herein, we propose a BSA approach as a combined chemotherapy that targets two signalling pathways as a way to achieve higher efficiency and avoid chemoresistance. We used a single, multifunctional but yet specific molecule, fluorenylmethoxycarbonyl-glucosamine-6-phosphate (FGlcP)4 to target two overexpressed proteins in cancer cells - alkaline phosphatase (ALP) and glucose transporter 1 (GLUT1) and to inhibit the glycolysis. We demonstrated the efficacy of the proposed approach in 2D cell culture5 and in spheroids6. In situ dephosphorylation of FGlcP by membrane-bound ALP (overexpressed in several cancers) triggered the ability of this carbohydrate amphiphile to self-assemble. This process induced a concentration and time dependent cell death in both cell cultures and microscopy observation revealed the formation of a nanonet entrapping cancer cells/spheroids. Inhibition of ALP rescued cells from death, confirming that ALP triggers BSA and activates apoptotic/necrotic pathways. In silico studies showed that FGlcP and its dephosphorylated analogue FGlc bind to glucose pocket in GLUT1 with higher affinity than glucose, suggesting a complementary blocking mechanism. This mechanism was confirmed in vitro by a competitive assay that confirmed the decrease of glucose uptake in the presence of either FGlcP or FGlc and by knockdown of GLUT1 that rescued the cells. In addition, the blockage of glucose uptake was also validated in vivo by positron emission tomography imaging. The treated with FGlcP spheroids were re-plated and we did not observe a relapse, thus, confirming the efficacy of the treatment. In conclusion, we report on a combinatorial approach based on in situ BSA and glycolysis inhibition that induces cell death selectively and efficiently and thus, is a promising chemotherapy against cancer.

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Biography

Alexandra Brito is currently a Pos-doctoral researcher in 3B's Research Group. She has a PhD in Tissue Engineering and Regenerative Medicine and Stem Cells from 3B's Research Group, University of Minho. Her research is based on the use of simple and minimalistic carbohydrate-based systems as new therapeutics, that incorporate bio-cues that are important for biological applications, including cancer. Her work is focused on studying mechanism pathways to better explain the effect of these compounds. These studies provide new light on the understanding of how healthy and pathological cells work, providing this way some new insights on finding new effective therapies.

OP08

Modelling lung cancer metastasis through a human microcirculation-on-a-chip

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Metastasis is a highly dynamic and multifactorial process characterized by the escape of cancer cells from the primary tumor to a secondary site through the microvasculature. Lymphatic vessel remodelling in the tumor microenvironment has been shown to facilitate cancer spread through the lymphatic system and promote cancer dissemination. Although several microfluidic models have already been reported to study metastasis formation, most platforms focus merely on cell invasion through the blood vessels[1,2].

This presentation will provide an overview of current microcirculation-on-a-chip models and highlight our recent work in developing a tumor-on-a-chip model, integrating both blood and lymphatic microvasculature. The model is currently being used to investigate the role of tumour-released mediators, particularly tumour-derived extracellular vesicles, in lung cancer metastasis and serve as a drug screening platform for selecting adequate therapy. Overall, the designed platform will constitute a powerful platform to unravel the mechanisms that drive cancer cell intravasation and permit personalized medicine.

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Biography

Catarina M. Abreu holds an integrated Master's degree in Biological Engineering from Instituto Superior Técnico (PT) and a PhD in Medicine from Swansea University Medical School (UK)in collaboration with the International Iberian Nanotechnology Laboratory. Her PhD work was dedicated to developing biosensors for the non-invasive identification of the most viable embryo for implantation during in vitro fertilization cycles. In this work, Catarina identified a correlation between molecular profiles and developmental competence of human embryos through the analysis of embryo-secreted molecules to the culture media. In 2020, Catarina joined the 3B's Research Group from the University of Minho (Portugal), as a world-recognized Institute in TERM and biomaterials, as a postdoctoral researcher to develop a new generation of tumor-on-chip models with integrated biosensors to study the role of the tumor microenvironment in cancer progression and metastasis.

Thank you for your participation!

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