FoReCaST

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

2D AND 3D IN VITRO TISSUE MODELS FOR DRUG SCREENING: STRENGTHS AND LIMITATIONS OF THE CANCER MODELS

Final Conference

27 – 29 October > Porto > Portugal

FoReCaST

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

CONFERENCE HIGHLIGHTS

The FoReCaST Final conference will take place from 27th to 29th of October 2021, in the very hospitable city of Porto, in Portugal.

FoReCaST Final Conference is organized by 3B's Research Group team focusing on 2D and 3D *in vitro* Tissue Models for Drug Screening: Strengths and Limitations of the Cancer Models. A range of world scientific leaders will be meeting and presenting their latest research, covering the most relevant topics related to nanotechnology in cancer research, 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 ~20 years in the fields of biomaterials sciences and tissue engineering to consolidate this new research topic.

In addition, besides a cutting-edge scientific program in the field of the Cancer Nanotechnology, we have included a dynamic social program that will allow an informal interaction between participants and a delightful experience in this beautiful historical city of Porto.

Some of the objectives of this conference 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:

- Biomaterials
- Biomimetics
- Scaffolds
- Hydrogels
- Spheroids
- 3D tumour models
- Organs-on-chip
- Microfluidics
- Bioinformatics
- Cell engineering
- Cell Signaling
- Clinical applications
- Translational Research
- Nanomedicine
- Nanotechnology
- Biosensing
- Drug Screening

The conference venue will be the Dr. António Cupertino de Miranda Foundation that was set up in 1964. This Foundation is located at "Avenida da Boavista", the longest avenue in Porto that is more than 5 km in length and crosses 6 neighbourhoods! Porto is one of the country's most beautiful and important historical cities. From the picturesque streets of downtown Porto to the churches and monuments, which offer spots of unrivalled beauty, meandering along romantic routes, cruises on the River Douro, visits to the port wine Cellars, the imposing presence of the Stock Exchange Palace, the architecture of Casa da Música or the Serralves Museum collection, there are many reasons why the city of Porto was classified as World Heritage by UNESCO as well as European BEST destination 2017!

Looking forward to meeting you in Porto!

Rui L. Reis
Conference Chair

Subhas C. Kundu
Conference Chair

SUPPORT

The FoReCaST Final conference 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:

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GENERAL INFORMATION

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

CONFERENCE CHAIR
Prof. Rui L. Reis
Prof. Subhas C. Kundu

LOCAL ORGANIZING COMMITTEE

Ana Guerra	Mariana Caldas
Ana Raquel Bastos	Mariana Carvalho
Banani Kundu	Miguel Neves
Cristiana Gonçalves	Nuno Neves
David Caballero	Ricardo Pires
Joaquim M. Oliveira	Rita Rebelo
Luisa Rodrigues	

CONFERENCE VENUE
The conference will be held at the Dr. António Cupertino de Miranda Foundation, in Porto (Portugal), at the Auditorium II.

Fundação Dr. António Cupertino de Miranda
Avenida da Boavista, 4245
4100-140 Porto
Portugal

Telephone (+351) 226 101 189
Fax (+351) 226 103 412

General e-mail: geral@facm.pt

GETTING TO AND FROM THE VENUE
You can use public transportation (bus no. 502 and 504) or private transportation (taxi) to get to the congress venue.

By car
From the south: Take A1 motorway in the direction of Porto, continue by Avenida da Boavista until Dr. António Cupertino de Miranda Foundation.

From the north: A28 motorway from Valença. A3 motorway from Braga. A7 motorway from Guimarães.

Once in Porto: Dr. António Cupertino de Miranda Foundation is located almost at the end of Avenida da Boavista (Boavista Avenue), in front of the Parque da Cidade (City Park). This avenue connects the ocean, near the famous "Castelo do Queijo", an awesome fortress near the beach, with the Rotunda da Boavista (Praça Mouzinho de Albuquerque), next to the beautiful "Casa da Música", a masterpiece designed by the Dutch and Pritzker Prize winner architect Rem Koolhaas.

By plane:
16 Km/20 minutes away from Francisco Sá Carneiro Airport (OPO).

GPS Coordinates: 41º 9' 54" N (Latitude), 8º 40' 24"W (Longitude)

Recommended Hotels to conference venue:

Crowne Plaza Hotel ***** Venue distance: 2.8 km
Porto Palácio Congress Hotel & Spa ***** Venue distance: 3.0 km
Sheraton Porto Hotel & Spa ***** Venue distance: 2.8 km
BessaHotel Boavista: Venue distance: 2.6 km
Hotel HF Fénix Porto **** Venue distance: 3.9 km
Hotel HF Ipanema Porto **** Venue distance: 4.2 km
Hotel da Música **** Venue distance: 3.9 km
Hotel HF Tuela Porto *** Venue distance: 4.0 km
So Cool Hostel Porto *** Venue distance: 4.5 km
Bluesock Hostels *** Venue distance: 6.2 km
Wine Hostel *** Venue distance: 5.8 km
Porto Alive Hostel *** Venue distance: 6.2 km
Happy Porto Hotel & Apartments *** Venue distance: 5.5 km

REGISTRATION AND INFORMATION DESK
All attendees must be registered for the conference. Admission to the conference is permitted only to those wearing the official conference 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 conference.

The information/registration desk will be located at the Foyer of the Auditorium II in the first day of the conference and will be open during the following days.

INTERNET
Wireless network at Dr. António Cupertino de Miranda Foundation will be available. Please address information desk to get the username and password, if necessary.

LUNCH AND DIETARY REQUIREMENTS
Lunch planned for the conference is included in the registration fee and will be served at the restaurant of the Dr. António Cupertino de Miranda Foundation Foundation. Please inform Organizing Secretariat at registration desk as soon as possible in case you have any dietary requirements.

SMOKING POLICY
From the 1st of 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 Venue building.

PHOTOGRAPHY POLICY
Recording and photographing conference 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 Porto, there is metro and bus that lets you travel through the city centre. If you want to travel by bus/metro, the tickets (andante) prices for one day are from 7,00€ on. The Bus 502 and 504 leave you in the Conference Venue, the stop is "Parque da Cidade".

Airport: www.ana.pt
Train: www.cp.pt
Metro: www.metrodoporto.pt
Bus: www.stcp.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 in the very heart of the city of Porto. Just go out and enjoy! To know more about the city, please visit the following websites:

www.portoturismo.pt
www.portoenorte.pt
www.lonelyplanet.com/portugal/the-north/porto/things-to-do

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, or during the social events. They are entitled to make any changes, modifications or omissions with respect to the information published in this book.

INSURANCE
The Conference Organisers cannot accept any responsibility for personal accidents and damage to the private property of Conference 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
25 minutes presentation
5 minutes discussion

SHORT ORAL PRESENTATION FORMAT
12 minutes presentation
3 minutes discussion

PRESENTATIONS UPLOAD
The conference presentations will all take place at the Auditorium II of Dr. António Cupertino de Miranda Foundation.

No personal computers will be allowed for the presentation. Files must be prepared in Power-Point 2007 or 2010 in a USB pen drive.

There will be a Speakers Preparation Desk identified in the information/registration desk in the ground floor where all speakers **MUST** upload their presentation as soon as possible with the deadline as in the following schedule:

SESSION	Upload deadline	
	Day	Deadline Time
Keynotes Presentations (27.10 | Afternoon)	27-Oct	14h00
Oral Presentations (27.10 | Afternoon)	27-Oct	14h00
Keynotes Presentations (28.10 | Morning)	27-Oct	16h00
Oral Presentations (28.10 | Morning)	27-Oct	16h00
Keynotes Presentations (28.10 | Afternoon)	28-Oct	09h30
Oral Presentations (28.10 | Afternoon)	28-Oct	10h30
Keynotes Presentations (29.10 | Morning)	28-Oct	16h00
Oral Presentations (29.10 | Morning)	28-Oct	16h00
Keynotes Presentations (29.10 | Afternoon)	29-Oct	09h30
Oral Presentations (29.10 | Afternoon)	29-Oct	10h30

POSTER PRESENTATIONS

Posters will be presented as E-Posters. All posters will be available for viewing in the screen before, during and after the conference. Discussion will be possible during the breaks scheduled in the program.

CONFERENCE PROGRAM

	Day 1 | Wednesday October 27 Auditorium II	Day 2 | Thursday October 28 Auditorium II	Day 3 | Friday October 29 Auditorium II
09.00 | 09.15		**KL3 - Luca Primo** *The University of Torino, Italy*	**KL8 - Karla Queiroz** *Mimetas, The Netherlands*
09.15 | 09.30			
09.30 | 09.45			
09.45 | 10.00			
10.00 | 10.15		SOP5 - Carlos Guimarães	SOP9 - Lara Pierantoni
10.15 | 10.30		SOP6 - Azin Khodaei	SOP10 - Daniel Mendanha
10.30 | 10.45		*Coffee-Break | Poster Session*	*Coffee-Break | Poster Session*
10.45 | 11.00			
11.00| 11.15		**KL4 -Jai Prakash** *University of Twente, The Netherlands*	**KL9 - Kristian Pietras** *Lund University, Sweden*
11.15| 11.30			
11.30| 11.45			
11.45| 12.00			
12.00| 12.15		*OP1- Talya Dayton*	*OP2 - Danielle Baptista*
12.15| 12.30			
12.30 | 14.30	*Lunch*	*Lunch*	*Lunch*
14.30 | 14.45	***Opening Ceremony***	**KL5 - Yuval Shaked** *Israel Institute of Technology, Israel*	**KL10 - Bruno Costa e Silva** *Champalimaud Foundation, Portugal*
14.45 | 15.00	**KL1- Lance Munn** *Harvard Medical School, USA*		
15.00 | 15.15			
15.15 | 15.30			
15.30 | 15.45		**KL6 - Eduard Batlle** *Institute for Research in Biomedicine, Spain*	SOP11 - Mariana Carvalho
15.45 | 16.00	SOP1 - David Cabalero		SOP12 - Catarina Oliveira
16.00 | 16.15	SOP2 - Sara Amorim		SOP13 - Mariana Caldas
16.15| 16.30	*Coffee-Break | Poster Session*		*Coffee-Break | Poster Session*
16.30 | 16.45		*Coffee-Break | Poster Session*	
16.45 | 17.00	***KL2 – Paula Oliveira*** *UTAD, Portugal*	**KL7 - Farshid Guilak** *Washington University, USA*	**KL11 - Celso Reis** *Institute for Research and Innovation in Health, Portugal*
17.00 | 17.15			
17.15 | 17.30			
17.30 | 17:45			
17.45 | 18:00	SOP3 - Banani Kundu		***Closing Ceremony***
18.00 | 18:15	SOP4 - Daniel Rodrigues	SOP7 - Miguel Neves	
18.15 | 18:30		SOP8 - Alexandra Brito	

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

	Day 1 | Wednesday, Oct 27th
09.00-12.00	Registration
14.30-14.45	**Welcome and Opening Ceremony** Rui L. Reis and Subhas C. Kundu *(3B's Research Group, University of Minho, Portugal | FoReCaST 2021 Conference Chairs)*
	Chair: Rui L. Reis | Co-Chair: S. C. Kundu
14.45-15.45	**KL1 - *In Vitro* Systems for Deconvolving Tumor Dynamics** Lance L. Munn *(Department of Radiation Oncology, Massachusetts General Hospital / Harvard Medical School, Boston, United States)*
	Short Oral Presentations
15:45-16:00	**SOP1 - Protrusion Fluctuations as a Predictive Morphodynamic Signature of Tumor Invasion** D. Caballero, C. M. Abreu, A. C. Lima, V. Brancato, N. M. Neves, V. M. Correlo, J. M. Oliveira, R. L. Reis, S. C. Kundu *(3B's Research Group, University of Minho, Portugal)*
16:00-16:15	**SOP2 - Hyaluronan of Low Molecular Weight Triggers the Invasive "Hummingbird" Phenotype on Gastric Cancer Cells** S. Amorim, D. Soares da Costa, I. Pashkuleva, C. A. Reis, R. L. Reis, R. A. Pires *(3B's Research Group, University of Minho, Portugal)*
16.15-16.45	*Coffee-Break | Poster Session*
	Chair: Miguel Oliveira
16.45-17.45	**KL2 - Animal models in cancer research: their strengths and limitations** Paula A. Oliveira *(Centre for Research and Technology of Agro-Environmental and Biological Sciences, Inov4Agro, University of Trás-os-Montes and Alto Douro, Portugal)*
	Short Oral Presentations
17:45-18:00	**SOP3 - Matrix Stiffness - The Manipulator of Cell Behaviour** B. Kundu, V. Brancato, J. M. Oliveira, V. M. Correlo, R. L. Reis, S. C. Kundu *(3B's Research Group, University of Minho, Portugal)*
18:00-18:15	**SOP4 - Laser Ablation Triggers EMT Associated Translational Response in Melanoma Tumor Spheroids** D. B. Rodrigues, D. Cruz-Moreira, R. L. Reis, R. P. Pirraco *(3B's Research Group, University of Minho, Portugal)*

	Day 2 | Thursday, Oct 28th
	Chair: Ricardo Pires
09.00-10.00	**KL3 - Three-dimensional cancer cell models in preclinical research** Luca Primo *(Department of Oncology - University of Torino - Torino, Italy)*
	Short Oral Presentations
10.00-10.15	**SOP5 - Hydrogel Bioarchitectures for Modelling and Digitalizing 3D Cancer Dynamics Toward the Discovery of Functional Drug Thresholds** C. F. Guimarães, L. Gasperini, R. Ahmed, A. P. Marques, U. Demirci, R. L. Reis *(3B's Research Group, University of Minho, Portugal)*
10.15-10.30	**SOP6 - Colloidal and Fibrous Thermo/Magnetic-Responsive Platforms to Deliver Curcumin as an Antineoplastic Agent** A. Khodaei, F. Jahanmard, R. Bagheri, H.R. Madaah Hossini, A. Dabbagh, S. Amin Yavari *(Institute for Nanoscience and Nanotechnology, Sharif University of Technology, Tehran, Iran)*
10.30-11.00	*Coffee-Break | Poster Session*
	Chair: Manuela Gomes
11.00-12.00	**KL4 - Translating the tumour microenvironment in three dimensional (3D) models** Jai Prakash *(Engineered Therapeutics group, Department of Biomaterials, Science and Technology, University of Twente, The Netherlands)*
	Oral Presentations
12.00-12.30	**OP1 - Organoid Models Reveal Pathways Important for Neuroendocrine Cell Growth, Differentiation, and Transformation** Talya L. Dayton, Nicolas Alcala, Lisanne Den Hartigh, Laura Moonen, Lise Mangiante, José Luis McFaline-Figueroa, Sonja Levy, José van den Berg, Jules Derks, Rachel S. van Leeuwaarde, Anne-Marie Dingemans, Niels Kok, Wieneke Buikhuisen, Koen Hartemink, Ernst Jan Speel4, Gerlof D. Valk, Margot E. Tesselaar, Menno R. Vriens, Susana M. Chuva de Sousa Lopes, Matthieu Foll, Lynnette Fernandez-Cuesta, Hans Clevers *(Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences, Utrecht, The Netherlands)*
12.30-14.30	*Lunch (Restaurant)*
	Chair: Vitor Correlo
14.30-15.30	**KL5 - The plasticity of stromal cells at the tumour microenvironment and their contribution to tumour fate** Yuval Shaked *(Rappaport-Technion-Integrated Cancer Center, Rappaport Faculty of Medicine, Technion - Israel Institute of Technology, Israel)*
15.30-16.30	**KL6 - LGR5+ cancer cells in chemotherapy resistance and metastasis** Eduard Batlle *(Institute for Research in Biomedicine (IRB Barcelona) and ICREA, Barcelona)*
16.30-17.00	*Coffee-Break | Poster Session*

	Chair: Tiago Silva
17.00-18.00	**KL7 - Engineering the genome to develop new biologic therapies for arthritis** Farshid Guilak *(Department of Orthopaedic Surgery, Washington University in St. Louis MO and Shriners Hospitals for Children – St. Louis, USA)*
	Short Oral Presentations
18.00-18.15	**SOP7- Novel Surface- and Biochemistry Platforms for Cancer Biosensing, Therapy and Beyond.** M. A.D. Neves, M. E. Caldas, R. N. Rebelo, A. I. Barbosa, J. M. Oliveira, V. M. Correlo, R. L. Reis, S. C. Kundu *(3B's Research Group, University of Minho, Portugal)*
18.15-18.30	**SOP8 – O-Glycotripeptides as Minimalistic Molecular Models of Glycoproteins** A. Brito, D. Dave, A. Lampel, V. I. B. Castro, D. Kroiss, R. L. Reis, T. Tuttle, R. V. Ulijn, R. A. Pires, I. Pashkuleva *(3B's Research Group, University of Minho, Portugal)*

	Day 3 | Friday, Oct 29th
	Chair: Nuno Neves
09.00-10.00	**KL8 - Advances in tumor modeling for cancer drug development** Karla Queiroz *(MIMETAS BV, Oegstgeest, The Netherlands)*
	Short Oral Presentations
10.00-10.15	**SOP9 - Silk Fibroin-based 3D *In Vitro* Breast Cancer Model for Drug Screening Applications** L. Pierantoni, V. Brancato, J. B. Costa, S. C. Kundu, R. L. Reis, J. Silva-Correia, J. M. Oliveira *(3B's Research Group, University of Minho, Portugal)*
10.15-10.30	**SOP10 - A New Chalcone Derivative for Glioblastoma Treatment** D. Mendanha, J. V. de Castro, J. Moreira, B. M. Costa, H. Cidade, M. Pinto, H. Ferreira, N. M. Neves *(3B's Research Group, University of Minho, Portugal)*
10.30-11.00	***Coffee-Break | Poster Session***
	Chair: Alexandra Marques
11.00-12.00	**KL9 - Exploring and exploiting functional subsets of breast cancer-associated fibroblasts** Kristian Pietras *(Division of Translational Cancer Research, Department of Laboratory Medicine, Lund University)*
	Oral Presentations
12.00-12.30	**OP2 - Realism vs simplicity - the bioengineering paradox in organ-on-chip systems** D. Baptista, Z. Tahmasebi Birgani, P. Habibović, L. Schurgers, B. Mees, S. Giselbrecht, R. Truckenmüller *(MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands)*
12.30-14.30	***Lunch (Restaurant)***
	Chair: Rogério Pirraco
14.30-15.30	**KL10 - Exosomes as emerging players in cancer biology and diagnostic applications** Bruno Costa-Silva *(Champalimaud Research, Champalimaud Centre for the Unknown, Portugal)*
	Short Oral Presentations
15.30-15.45	**SOP11 - Gastrointestinal Organoids-on-Chip: Challenges and Future Trends** M. R. Carvalho, L. Yan, Bo Li, C. Zhang, Y. He, R. L. Reis, J. M. Oliveira *(3B's Research Group, University of Minho, Portugal)*
15.45-16.00	**SOP12 - Marine-based Nanaoparticles with ERBB-2 Antibody Immobilized Target Breast Cancer Cells Both *in vitro* and *in vivo*** C. Oliveira, C. S. Gonçalves, E. P. Martins, N. M. Neves, R. L. Reis, Bruno M. Costa, T. H. Silva, A. Martins *(3B's Research Group, University of Minho, Portugal)*

16.00-16.15	**SOP13 - Melanin Nanoparticles as a Theranostic Approach for Cancer Treatment** M. Caldas, M. Neves, R. Rebelo, S. C. Kundu, R. L. Reis, V. M Correlo *(3B's Research Group, University of Minho, Portugal)*
16.15-16.45	*Coffee-Break | Poster Session*
	Chair: David Caballero
16.45-17.45	**KL11 - Glycosylation in cancer: molecular characterization and implications for cancer therapy** Celso A. Reis *(i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal)*
17.45-18.00	Closing ceremony


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POSTER LIST

	POSTERS LIST
P01	**DEVELOPMENT OF HIERARCHICAL SCAFFOLDS FOR BONE TISSUE ENGINEERING APPLICATIONS** R. Lemos, F. R. Maia, V. P. Ribeiro, J. B. Costa, P. J. G. Coutinho, R. L. Reis, J. M. Oliveira (3B's Research Group, University of Minho, Portugal)
P02	**ENGINEERING SILK FIBROIN/CHOLINIUM GALLATE BASED SPONGES ENVISIONING INFLAMMATORY DISEASES TREATMENT** J. M. Gomes, S. S. Silva, R. L. Reis (3B's Research Group, University of Minho, Portugal)
P03	**DIFFERENT APPROACHES TOWARDS EFFECTIVE CURCUMIN-BASED ANTIMICROBIAL PHOTOTHERAPY** E. Silva, I. Aroso, J. M. Silva, R. L. Reis (3B's Research Group, University of Minho, Portugal)
P04	**SHARK COLLAGEN AND CHONDROITIN SULFATE HYDROGELS ENVISAGING CARTILAGE TISSUE ENGINEERING** E. Martins, R. L. Reis, T. H. Silva (3B's Research Group, University of Minho, Portugal)
P05	**ACEMANNAN-BASED TERNARY BLENDED FILMS: A NATURAL APPROACH ENVISIONING BIOMEDICAL PURPOSES** L. C. Rodrigues, E. M. Fernandes, S. S. Silva, R. L. Reis (3B's Research Group, University of Minho, Portugal)
P06	**UNCONVENTIONAL SECRETION OF RHAMM BY BREAST CANCER CELLS** D. Soares da Costa, A. M. Carvalho, R. L. Reis, I. Pashkuleva (3B's Research Group, University of Minho, Portugal)
P07	**BIOACTIVITY SCREENING OF CYANOBACTERIA FOR IDENTIFICATION OF NOVEL ANTICANCER COMPOUNDS** A. C. Carvalho, C. Oliveira, E. M. Fernandes, A. Martins, P. N. Leão, V. M. Vasconcelos, R. L. Reis, T. H. Silva (3B's Research Group, University of Minho, Portugal)
P08	**INVESTIGATING THE ROLE OF CELL ADHESIVE CUES IN TUMOR PROGRESSION USING GELLAN GUM-BASED TISSUE ENGINEERED 3D OSTEOSARCOMA MODEL** N. Antunes, B. Kundu, L. P. Silva, S.C. Kundu, V. M. Correlo, R. L. Reis (3B's Research Group, University of Minho, Portugal)
P09	**OSTEOGENIC LITHIUM-DOPED BRUSHITE CEMENTS FOR BONE REGENERATION** S. Pina, K. Hurle, F.R. Maia, V.P. Ribeiro, J.M. Oliveira, F. Goetz-Neunhoeffer, R.L. Reis (3B's Research Group, University of Minho, Portugal)
P10	**NANOPATTERNED AND PEDOT-CONTAINING SILK FIBROIN NERVE GUIDANCE CONDUITS FOR PERIPHERAL NERVE REPAIR** A. Escobar, R.L. Reis, T.H. Silva, M.N. Collins, J.M. Oliveira (3B's Research Group, University of Minho, Portugal)
P11	**OPTIMIZATION OF THE DECELLULARIZATION OF FEEDER LAYERS AS A SUBSTRATE FOR THYMIC CELL CULTURE** C. S. Silva, R. D. Pinto, R. A. Pires, P. Ferreirinha, M. Correia-Neves, R. L. Reis, N. L. Alves, A. Martins, N. M. Neves (3B's Research Group, University of Minho, Portugal)
P12	**GALLIC ACID-BASED COMPOUNDS AS MODULATORS OF THE SUPRAMOLECULAR ASSEMBLY OF AMYLOID β PEPTIDE IN ALZHEIMER DISEASE** A. R. Araújo, J. Correa, V. Dominguez-Arca, R. L. Reis, E. Fernandez-Megia, R. A. Pires (3B's Research Group, University of Minho, Portugal)
P13	**STEM CELL-BASED ADVANCED THERAPY FOR NEURODEGENERATIVE DISEASES** H. Ferreira, D. Amorim, A. C. Lima, I. Laranjeira, R. P. Pirraco, A. R. Costa-Pinto, R. Almeida, A. Almeida, R. L. Reis, F. Pinto-Ribeiro, N. M. Neves (3B's Research Group, University of Minho, Portugal)
P14	**INFLUENCE OF THERMAL CONDITIONS IN THE 3D MICROARCHITECTURE OF THE SPONGY-LIKE HYDROGELS** J. Pires, L. P. da Silva, R. L. Reis, A. P. Marques (3B's Research Group, University of Minho, Portugal)

P15	**NATURAL-BASED ELECTRIC-RESPONSIVE GELLAN GUM BIOINKS FOR MUSCLE REPAIR** Y. H. Youn, L. P. da Silva, O. Alheib, I. K. Kwon, R. L. Reis, V. M. Correlo (3B's Research Group, University of Minho, Portugal)
P16	**MICROGUIDED LASER ABLATION FOR THE BIOFABRICATION OF SKIN SUBSTITUTES WITH FOLLICULAR UNITS** C.M. Abreu, L. Gasperini, M. E. L. Lago, R. L. Reis, A. P. Marques (3B's Research Group, University of Minho, Portugal)
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KEYNOTE LECTURES

KL1

In Vitro Systems for Deconvolving Tumor Dynamics

Lance L. Munn

Department of Radiation Oncology, Massachusetts General Hospital / Harvard Medical School

During tissue development, the collective organization single cells results in well-defined compartments that are separated by physical and biochemical barriers. This organization establishes the correct microanatomy (the spatial relationships between cells and with matrix components) that is maintained in the adult tissue, but often disrupted in diseases such as cancer. The self-assembly and homeostasis of the tissue structures are not only guided by biochemical pathways, but also by mechanical signals transmitted through structural components and fluids. Unfortunately, little is known about the mechanisms used by cells to participate in coordinated, collective behavior, or how these mechanisms fail in pathologies. Using in vitro assays, microfluidic devices, mathematical models and tissue engineering, we investigate how cells in various mechanical and chemical environments interact and cooperate to accomplish such varied goals as cancer invasion, morphogenesis and regulation of vascular function.

For example, forces exerted by flowing fluids provide important signals for the vascular endothelium. Blood flow exerts shear stresses on vessel wall cells that drive blood vessel contraction or dilation to optimize flow through a network, and plasma exchanged between vessels can coordinate sprouting angiogenesis. Mechanical signals can also affect tissue microanatomy: compressive forces created by growing tumors can induce polarization and collective migration of the cancer cells. These processes involve the integration of mechanical and chemical signals and the collective behavior of cells, and represent potential new targets for controlling tumor progression and vascular function.

Biography

Dr. Munn received his PhD in Chemical Engineering from Rice University in 1993. He is currently Associate Professor, Director of the Bioengineering, Microscopy and Computing Core, and Deputy Director of the Steele Lab for Tumor Biology at MGH. Dr. Munn has been studying vascular biology in the context of tumor physiology for the past 20 years, with a focus on vessel microanatomy and biomechanics. His group uses a combination of in vivo techniques, mathematical modeling and microfabrication to determine how cells cooperate to form and maintain tissues. Using these approaches Dr. Munn's group has identified and characterized the role of mechanobiology in tumor invasion, lymphatic drainage and neovascularization.

KL2

Animal models in cancer research: their strengths and limitations

Paula A. Oliveira

Centre for Research and Technology of Agro-Environmental and Biological Sciences (CITAB), Inov4Agro, University of Trás-os-Montes and Alto Douro (UTAD), Portugal

The laboratory rat (Rattus norvegicus) was the first mammal domesticated for scientific purposes and the first research study carried out with this species dating back to the 19th century. It has been possible to study the evolution of various diseases, develop and evaluate new diagnostic methodologies, and test new therapeutic approaches through animal models. Thus, thanks to their use, human life expectancy has been increasing.

This talk will be reviewed and discussed the principal characteristics of the most used animal models available to investigate cancer (urinary bladder, liver, mammary, prostate, liver, colorectal and skin). Considering that each model has its requirements, its strengths, limitations, and future directions will be presented, bearing in mind the experiences accumulated in analyzing their practical implementation and European directives.

Biography

Paula A. Oliveira (PAO) graduated in 1996 in Veterinary Medicine, at University of Trás-of-Montes and Alto Douro (UTAD), completed in 2001 a master's degree in Oncology from the University of Porto, and in 2006 a PhD in veterinary sciences at UTAD. Her PhD thesis was developed at UTAD, University of Santiago de Compostela (Spain) and Portuguese Institute of Oncology. PAO is habilitated in Veterinary Sciences since 2010. Her research focuses from the beginning in the implementation and analysis of animal models to study the bio-pathological mechanisms, to evaluate new therapies, to analyze chemoprevention measures and the interference of lifestyles in cancer development. PAO has published more than 150 articles on this subject.

KL3

Three-dimensional cancer cell models in preclinical research

Luca Primo

Department of Oncology - University of Torino - Torino, ItalyCandiolo Cancer Institute - Candiolo, Italy

The past few decades have seen a greater understanding of the molecular and genetic underpinnings of tumor etiology. However, indolent disease, metastatic colonization, dormancy, relapse, immune response and the rapid evolution of drug resistance are inadequately addressed using standard molecular and genetic characterization and standard monolayer cell culture models. New technology has been developed that allows tumor samples isolated from animal models and patients, to be maintained and grown in a 3D environment. These in vitro growing tumors, called tumor organoids, provide an unprecedented opportunity to combine genetic, phenotypic and functional studies in samples from individual patients. Tumor organoids appear to recapitulate genetic and morphological properties of the original tumor and therefore allow studies of sensitivity and resistance to a large number of anticancer drugs. Furthermore, organoids allow studying real-time dynamics of cancer cells and are readily amenable to live microscopy techniques, thereby overcoming many significant limitations of current preclinical models. The knowledge acquired with these 3D culture models, in terms of drug response or tumor aggressiveness, in future might be translated into therapeutic indications as well as for patient stratification.

Biography

Dr. Luca Primo is a full Professor of Biochemistry at the School of the Medicine University of Torino and a group leader at Candiolo Cancer Institute, where he coordinates the research activity of a small group of researchers, post-docs and PhD students. He has gained considerable experience in vascular cell biology and, more recently, in 3D cell cultures and tumour organoids. He started his research experience during his degree in Biology, characterizing the thrombospondin receptor CD36. Then, during his PhD in biochemistry, he focused his research efforts on the PI3K signalling in angiogenesis and cell motility. He discovered the role of PDK1 in cell motility, breast cancer growth and tumour invasion. In the angiogenesis field, He showed the regulatory mechanisms of integrins in endothelial cells and the role of endothelial podosomes in angiogenesis. Now, He is working on 3D culture models (cells spheroids, tumour organoids and tissue explants) to study collective motility and cell dynamics.

KL4

Translating the tumour microenvironment in three dimensional (3D) models

Jai Prakash

Engineered Therapeutics group, Department of Biomaterials, Science and Technology, University of Twente, The Netherlands

The physical and biochemical characteristics of the tumour microenvironment (TME) control cancer cell differentiation, proliferation, invasion, and metastasis. Cells surrounding cancer cells such as cancer-associated fibroblasts, macrophages, and other immune cells secrete factors that interact with cancer cells and stimulate their proliferation and migration. Furthermore, the noncellular component, such as the extracellular matrix (ECM), controls tumorigenesis and metastasis. There is a rush to develop new therapeutics to intervene in the crosstalk between cancer cells and TME. The 2D cultures are too simple, and the in vivo models are too complex to understand the complexity of the TME interactions. It is therefore imperative to develop advanced in vitro models that mimic the TME. 3D in vitro models are widely used because they can incorporate different patient-derived tissues/cells and allow longitudinal readouts, thus permitting a deeper understanding of cell interactions. Therefore, these models are excellent tools to bridge the gap between oversimplified 2D systems and animal models. We have developed different 3D models to mimic various features of the TME concerning different cancer types such as breast cancer, glioblastoma, pancreatic cancer. We used other tools to generate these models, such as 3D hetero-spheroids for breast and pancreatic tumour models. These models are applied for evaluating anti-stromal agents in vitro and correlated with in vivo models. We have recently created the stromal features of a pancreatic tumour in vitro to emulate the tumour stroma interaction. In addition, we have also used advanced tools of the 3D bioprinting technique to mimic the tumour architecture and immune system infiltration. Altogether, our studies show enormous opportunities to develop different 3D models to simulate the TME, which could be used to understand the complex tumour biology and applied to study the effect of novel therapeutics.

Biography

Jai Prakash, professor at the University of Twente in the Netherlands, is a pharmaceutical and bioentrepreneurial scientist working in the field of engineered therapeutics. He obtained his masters in Pharmacology from All India Institute of Medical Sciences and then obtained his PhD (with honors) from the University of Groningen, the Netherlands. Earlier, he worked as vice president at BiOrion Technologies and assistant professor at Karolinska Institutet, Sweden, and then joined as tenure track professor at the University of Twente. He is also the founder and CSO of ScarTec Therapeutics, which is developing peptide therapeutics against fibrosis and cancer. He is also serving as the president of BeNeLux and France local chapter for the Controlled Release Society (CRS). His research interests are in designing therapeutic technologies to disrupt the crosstalk between tumor cells and stromal cells and in developing advanced 3D in vitro models to mimic the tumor microenvironment.

KL5

The plasticity of stromal cells at the tumour microenvironment and their contribution to tumour fate

Yuval Shaked

Rappaport-Technion-Integrated Cancer Center, Rappaport Faculty of Medicine, Technion – Israel Institute of Technology, Israel

The limited success of cancer therapy, especially in advanced metastatic disease, warrants a complete understanding of the nature of cancer cells and the factors that drive their proliferation and spread. Stromal cells in the tumour microenvironment significantly affect cancer cell characteristics and tumour fate. In this study, in particular, we focused on the contribution of myeloid cells to metastasis. While the reservoir of these cells in the bone marrow (BM) compartment is well appreciated, their education, immune memory, and differentiation pattern from uncommitted hematopoietic stem cells (HSCs) to tumour-supporting immune cells are not fully understood. Here we used single-cell RNA sequencing (scRNAseq) of HSCs. The scRNAseq data were integrated with a proteomic screen of the tumour secretome to reveal a potential cross-talk between immune and tumour cells. We demonstrate that metastatic tumours dictate a unique differentiation pattern of uncommitted HSCs towards a specific myeloid progeny. We found that this differentiation is driven by the IL-6/IL-6R axis, which is highly active in metastatic tumours, promoting the enrichment of metastasis-supporting immune cells. Our study reveals how tumour cells hijack HSCs towards a specific long-lived myeloid differentiation, thereby enabling the enrichment of metastasis-associated immune cells in the tumour microenvironment. We, therefore, suggest a global function of tumour cells in the commitment of immune cells to metastatic-supporting cells, which in turn contribute to tumour outgrowth and spread. Identifying the driver proteins contributing to this pro-metastatic process can serve as a basis for the development of new therapeutic strategies against aggressive cancers.

Biography

Yuval Shaked (PhD), is a Professor at the Rappaport Faculty of Medicine, Technion, Israel, and the director of the Rappaport-Technion Integrated Cancer Center (RTICC). He obtained his PhD from the Hebrew University and was trained as a post-doctoral at Sunnybrook, University of Toronto, Canada, where the focus of his research was on cancer biology and therapy. As an independent investigator from 2008, he pioneered the field of the host response to anti-cancer therapy. Prof. Shaked received many awards, including the Krill Prize from the Wolf Foundation, Youdim prize for Excellence in Cancer Research and Hershel prize for innovation. He received the European Research Council award. Prof. Shaked is an author of over 130 published studies and holds more than five approved patents. He is also an entrepreneur and founded two companies, OncoHost and RemedyCell.

KL6

LGR5+ cancer cells in chemotherapy resistance and metastasis

Eduard Batlle

Institute for Research in Biomedicine (IRB Barcelona) and ICREA, Barcelona.

The cancer stem cell (CSC) concept states that tumor growth, analogous to the renewal of healthy tissues, is fueled by small numbers of dedicated stem cells. It provides attractive explanations for the clinical behavior of cancers and inspires treatment strategies that specifically target CSCs, the 'beating heart' of the tumor. Over the past years, an avalanche of studies has identified CSCs by expression of individual marker genes, but these cells' nature and features remain largely uncharacterized. As a case of the example, LGR5 has been established as a bonafide marker of CSCs in gastrointestinal tumors. Many CRCs contain abundant LGR5+ cells, yet clonal analysis suggests that only a tiny proportion of these cells function as CSCs. It is also known that many CRCs have a few o no LGR5+ cells, yet these tumors may still exhibit a hierarchical organization. Here, I will discuss our recent work on the functional states adopted by LGR5+ cells in CRC and the role of this cell population in chemotherapy resistance and metastatic dissemination.

Biography

Eduard Batlle is an ICREA Professor and Chair of the Cancer Science program at the Institute for Research in Biomedicine (IRB) in Barcelona, Spain. His laboratory focuses on the mechanisms that drive colorectal cancer initiation and progression. Amongst other findings, his research initially identified the transcription factor Snail is a repressor of E-Cadherin gene expression during the Epithelial-to-Mesenchymal Transition, a role for EphB/ephrin signalling in intestinal cell positioning and the connection between the intestinal stem cell program and colorectal cancer. More recently, Batlle revealed a key role for TGF-beta signalling in stromal cells during metastatic colonization. His laboratory is currently focused on developing new prognostic and therapeutic tools for advanced colorectal cancer based on targeting the tumour microenvironment. His track record has been recognized through several awards/honours such as the Debiopharm Life Science Award (2006), Josef Steiner Cancer Research Award (2013), ERC Starting and Advanced Grants (2007, 2013, 2019), the Pezcoller foundation-EACR award (2014), the Lilly Foundation Award for Preclinical research (2016) and the King Jaime-I award (2021).

KL7

Engineering the genome to develop new biologic therapies for arthritis

Farshid Guilak

Department of Orthopaedic Surgery, Washington University in St. Louis MO and Shriners Hospitals for Children – St. Louis, USA

Arthritis represents a painful and debilitating family of joint diseases that is characterized by progressive degeneration of the articular cartilage; however, there are currently few disease-modifying treatments available. Our lab has focused on tissue engineering approaches for resurfacing entire joints using adult stem cells and biomimetic 3D woven fiber scaffolds. Furthermore, we have used combinations of gene therapy and tissue engineering to develop tissue replacements that possess the capability for biologic drug delivery. In recent years, the advent of synthetic biology and gene-editing methods such as CRISPR-Cas9 has allowed for precise modifying gene networks that control cell behavior. We have applied a combination of principles from these fields to rewire cellular gene circuits in stem cells in a manner that allows us to create a unique, custom-designed cell type that can sense and respond to its biochemical environment in a pre-programmed way. These cells have been used to develop engineered tissue replacements with tunable, inducible, or feedback-controlled, auto-regulated biological responses. We have recently developed synthetic "mechanogenetic" gene circuits that express therapeutic transgenes in response to defined mechanical signals using this approach. In addition to recapitulating the biochemical and biomechanical properties of the tissue, these "smart" cells and constructs can provide controlled drug delivery and immunomodulatory responses to the joint as therapies for cartilage repair or arthritis.

Biography

Dr Farshid Guilak is a Professor of Orthopaedic Surgery at Washington University, Director of Research for the St. Louis Shriners Hospitals for Children, and Co-director of the Washington University Center of Regenerative Medicine. His laboratory is pursuing a multidisciplinary approach for developing new tissue engineering and stem cell-based therapies for musculoskeletal diseases, spearheading new strategies that combine genome engineering and synthetic biology to stem cells. He has published over 370 articles in peer-reviewed journals and has co-edited four books. He is the editor-in-chief of the Journal of Biomechanics and serves on numerous other journal editorial boards. He has won several national and international awards for his research, including four separate awards for mentoring. He has also worked extensively in the translation of tissue engineering technologies. He is the Founder of Cytex Therapeutics, a startup company focusing on developing new regenerative medicine therapies for musculoskeletal conditions.

KL8

Advances in tumor modeling for cancer drug development

Karla Queiroz

MIMETAS BV, The Netherlands

Current *in vitro* tumour models fail to recapitulate the complexity of the tumour microenvironment. Consequently, early drug discovery in oncology does not account for the influence of the diverse bio- and physicochemical features of tumours that impact response to treatment. This also limits the ability to use tumour models for predicting clinical responses to anticancer drugs. Therefore, there is an urgent need for novel tumour models and modelling platforms. Here, I discuss the relevance of microfluidic technology for creating 3D models that are grown under flow conditions and the inclusion of relevant components of the tumour microenvironment. In addition, I will discuss how Mimetas has been developing robust assays to its models, enabling their application in high throughput phenotypic screening. We envision that OrganoPlate based tumour models will build a new paradigm in modelling interactions in the tumour microenvironment and its consequences for tumour aggressiveness and drug responses. We hope that these models will also evolve into powerful tools for patient stratification and treatment selection in the long term, enabling personalized medicine applications.

Biography

Dr. Karla Queiroz received her PhD in 2011 at the Erasmus University in Rotterdam (Netherlands). She worked on signal transduction and chemoresistance in tumour diseases. As a postdoctoral researcher, she worked from 2010-2015 at Amsterdam UMC/AMC in the department of molecular and experimental medicine in inflammation and cancer and at the Flemish Institute for Biotechnology in Leuven, the field of vascular biology. During her postdoctoral studies, she focused on the role of constituents of the tumour microenvironment in tumour progression. Currently, she works as a Senior Scientist to develop in vitro 3D-organ models at the biotech company Mimetas BV, The Netherlands.

KL9

Exploring and exploiting functional subsets of breast cancer-associated fibroblasts

Kristian Pietras

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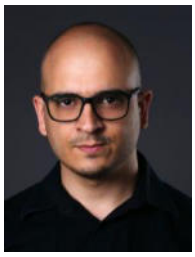
The overarching purpose of our work is to functionally define the cellular elements of tumors at single-cell resolution and elucidate their influence on malignant parameters with the conviction that drug targets and/or biomarkers may be identified. Our research follows three avenues, each focusing on a particular stromal cell type with a comprehensive approach integrating basic science, pre-clinical studies and translational efforts. Here, emphasis will be put on cancer-associated fibroblasts (CAFs), which are a prominent constituent of the tumor microenvironment. However, the origin of CAFs, and their role(s) in shaping disease initiation, progression and treatment response remain unclear due to significant heterogeneity within the population. We have improved the resolution of the widely defined CAF population by utilizing single cell RNA sequencing, which has opened the possibility for biomarker-driven development of drugs for precision targeting CAFs. As a case-in-point, we have recently delineated a previously unappreciated role for CAFs as determinants of the molecular subtype of breast cancer. Pharmacological intervention of the crosstalk between basal-like breast cancer cells and a specific subset of CAFs results in the conversion of the malignant tissue into a hormone receptor-positive state that enhances sensitivity to endocrine therapy.

We continually strive to transform fundamental knowledge into guiding principles for the clinical development of new prognostication or treatment of malignant diseases. Our conviction is that the stromal compartment comprises a hitherto underexploited source for factors harboring prognostic, predictive or therapeutic potential.

Biography

Kristian Pietras was awarded a Ph.D. in 2002 at the Ludwig Institute for Cancer Research, Uppsala University, Sweden. Pietras performed his postdoctoral work at the University of California, San Francisco, in the laboratory of Douglas Hanahan. Upon his return to Sweden and Karolinska Institutet, Pietras developed his independent line of research. In 2012, he was recruited as the Grosskopf Professor to Lund University, where he acted as the Director of Lund University Cancer Centre between 2015-2021. Pietras has made important contributions in defining tumors as communicating organs comprising multiple cell types that collectively sustain cancer progression as a well-positioned link between basic science, oncology and the pharmaceutical industry. Pietras has received numerous awards for his research, most notably the Anders Jahre's Medical Prize for young scientists by Oslo University. The Fernström Award for Young Scientist by Lund University and the Göran Gustafsson Award by the Royal Swedish Academy of Sciences. He has been a member and chairman of the Young Academy of Sweden.



KL10

Exosomes as emerging players in cancer biology and diagnostic applications

Bruno Costa-Silva

Champalimaud Research, Champalimaud Centre for the Unknown, Portugal

Extracellular vehicles (EVs), membrane vesicles released by all cells, are emerging mediators of cell-cell communication. By carrying biomolecules from tissues to biofluids, EVs have attracted attention as non-invasive sources of clinical biomarkers in liquid biopsies. We here present a flow cytometry (FC) strategy that reduce biofluids processing time, costs and volume requirements by not requiring isolation or concentration of EVs prior to staining. We illustrate its application to monitor tumor-associated EVs populations in metastatic pancreatic cancer patients. We also show unpublished work on the identification of EVs markers of liver metastatic stroma in pancreatic cancers.

Biography

Dr. Bruno Costa-Silva obtained his PhD in Oncology at the Ludwig Institute for Cancer Research and AC Camargo in São Paulo, Brazil, working on pre-metastatic niches induction by tumor-derived EVs. Then he moved to Weill Cornell Medical College in New York for his post- doc at Dr. Lyden´s laboratory, where he spearheaded studies on the role of EVs in cancer pathogenesis, specifically in metastasis of pancreatic cancer. Since 2016 he moved to Lisbon where he is leading the Systems Oncology group at the Champalimaud Foundation. He currently leads projects on the EVs role on the setup of tumor microenvironment.

KL11

Glycosylation in cancer: molecular characterization and implications for cancer therapy

Celso A. Reis

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Alterations of glycosylation are common molecular alterations with significant biological implications for cancer progression. Cancer is a heterogeneous disease that requires multidisciplinary treatment. Current targeted therapy depends on patient stratification based on the molecular features of the tumor. This presentation will report based on alterations of glycosylation that occur in cancer. Recent results apply glycomic and glycoproteomic strategies in human cancer that provided novel information with significant clinical implications. This presentation will report on the alterations of glycosylation impact the activation of oncogenic receptors in tumour samples, advanced cell and tissue models, including the recently described glycoproteomic map of the HER2 (ErbB2) in gastric cancer cells. Glycomic and glycoproteomic analysis of HER2 disclosed a site-specific glycosylation profile in gastric cancer cells. HER2-specific glycosylation alters the cellular half-life of this receptor and the sensitivity of HER2-dependent gastric cancer cells to trastuzumab-induced cytotoxicity. These results disclose novel functional aspects of glycosylation modifications occurring in cancer and highlight their potential for patient stratification, personalize medicine and novel and improved therapeutic applications.

Biography

Celso A. Reis is the Head of the Glycobiology in Cancer group at i3S - Institute for Research and Innovation in Health, University of Porto. He leads an international multidisciplinary team on Glycobiology in human diseases with a focus on cancer. Celso has published 211 peer-reviewed papers and several patents, with over 12700 citations and an H-index of 53 (Scopus). ORCID: https://orcid.org/0000-0002-0286-6639 His research focuses on the role of glycosylation in human cancer. He has addressed the molecular mechanisms controlling glycosylation in cancer cell biology, specifically the role of glycosyltransferases regulating the biosynthesis of glycans in cancer. These included the role of sialyltransferases leading to the biosynthesis of terminal sialylated glycan antigens expressed by cancer cells. He also contributed to seminal studies determining the role of these glycosylation modifications and the consequences to cancer cell biology, including the mechanisms regulating cancer invasion, metastasis and tumour glycoimmunology. Celso A. Reis has also contributed to applying glycoconjugates as biomarkers in human cancer and pre-neoplastic lesions and applying novel unique therapeutic strategies. He currently develops novel approaches to improve cancer diagnosis prognosis and improve cancer treatment based on glycans. They can be used as markers for cancer patient stratification and glycans and glycoconjugates that can be used as novel targets in advanced cancer therapy strategies.

NOTES

ORAL PRESENTATIONS

OP1

ORGANOID MODELS REVEAL PATHWAYS IMPORTANT FOR NEUROENDOCRINE CELL GROWTH, DIFFERENTIATION, AND TRANSFORMATION

Talya L. Dayton[1,2], Nicolas Alcala[3], Lisanne Den Hartigh[1,2], Laura Moonen[4], Lise Mangiante[3], José Luis McFaline-Figueroa[5], Sonja Levy[6], José van den Berg[6], Jules Derks[4], Rachel S. van Leeuwaarde[7], Anne-Marie Dingemans[8], Niels Kok[6], Wieneke Buikhuisen[6], Koen Hartemink[6], Ernst Jan Speel[4], Gerlof D. Valk[7], Margot E. Tesselaar[6], Menno R. Vriens[7], Susana M. Chuva de Sousa Lopes[9,10], Matthieu Foll[3], Lynnette Fernandez-Cuesta[3], Hans Clevers[1,2,11]

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[11] Princess Máxima Centre, The Netherlands

A paucity of preclinical models has a limited study of neuroendocrine (NE) cell-derived tumors, Neuroendocrine neoplasms (NENs). A little is known about normal NE cells and how they contribute to NEN formation. We have applied the organoid culture system to study neuroendocrine cells and tumors. To study NENs, we generated patient-derived tumor organoids (PDTOs) of rare, high-grade NENs from different tissue sites: the lung, the gastroenteropancreatic (GEP) system, and the skin. We also generated the first PDTOs of low-grade NENs from the lung and intestine. Genomic analysis of NEN PDTOs and matched primary tumors show that PDTOs recapitulate the molecular features of the primary tumor and maintain intratumor heterogeneity. Whereas high-grade NENs readily form PDTOs that can be expanded consistently, PDTOs of low-grade NENs are difficult to generate and have exceedingly slow growth rates. This recapitulates what is observed for these NEN subtypes in patients. By modifying NEN PDTO growth media, we have defined small molecule components that promote or inhibit NEN cell growth in culture, suggesting potential therapeutic vulnerabilities that we are currency assessing through targeted drug screen assays. In complementary experiments, we developed an organoid system to study normal NE cells of the lung. Using single-cell RNA-seq throughout induced NE cell differentiation, we resolve the transcriptional changes associated with NE cell differentiation and further define this rare but physiologically important cell type.

Biography

Talya L. Dayton is a senior postdoctoral fellow in Hans Clevers' Laboratory at the Hubrecht Institute, Utrecht, the Netherlands. In her research, she is using organoids to study neuroendocrine cells and tumors. In 2016, Talya received a Ph.D. from the Massachusetts Institute of Technology (MIT) for her work in the lab of Tyler Jacks using mouse models to study metabolism in development and cancer. Talya was awarded fellowships from the National Science Foundation and the Department of Defense for her graduate work. Talya has been awarded an EMBO Fellowship, a Marie Curie Individual Postdoctoral Fellowship, and has received funding from the Neuroendocrine Tumor Research Foundation (NETRF) for her postdoctoral work.

OP2

Realism vs simplicity – the bioengineering paradox in organ-on-chip systems

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There is a growing demand for Organ-on-chip (OoC) systems for research with potential applications in academia, industry and the clinic. Their applicability ranges from stem cell research, cancer research and developmental biology over tissue replacement and bioartificial organs to toxicity and pharmaceutical drug testing. Organ-on-chip (OoC) technology promises a customizable system that can harness critical functional aspects of target tissues and organs in a controlled and automated manner. The bioengineering paradox resides in the fact that, when designing such systems, it is virtually impossible to increase realism without compromising on simplicity. There is a constant pursuit of authenticity and clinical relevance. Yet by trying to recapitulate every physical, chemical and biological feature of the cellular microenvironment of a certain tissue or organ, the system becomes substantially more complex and user-unfriendly and unreliable. In contrast, oversimplification of the system may lead to neglecting microenvironmental key factors that are indispensable. A recurrent strategy to navigate within this overwhelming universe of possibilities is to engineer its basic functional unit, aiming to recapitulate the organ's essential functions. In this talk, we will explore recent advances of OoC systems that offer the right balance between complexity and simplicity by the example of membrane/film-based human lung and coronary artery models.

Biography

Danielle Baptista has a background in Biomedical Engineering and Biophysics. For her Master's, she specialized in Clinical Engineering and Medical Instrumentation, where she discovered Tissue Engineering and Regenerative Medicine. Intense curiosity for biomedical matters and a challenge-driven personality led her to pursue, in 2015, a PhD in this field at MERLN Institute in Maastricht, where Danielle now continues as a Post-doc. Danielle's work focuses on developing microengineered biomimetic *in vitro* models for different organs and research applications, where she combines biology, microfabrication and microfluidics. She hypothesizes that the native microenvironment should be considered an essential feature when designing realistic *in vitro* models. Danielle extends her work from the development of biomimetic alveolar and bronchial lung models to platforms for lung organoid culture and micromanipulation using microthermoforming and lithography techniques. Recently, she joined a project to develop a coronary artery-on-chip model to investigate arterial calcification and decalcification strategies to be implemented in a clinical setting.

SHORT ORAL PRESENTATIONS

SOP1

Protrusion Fluctuations as a Predictive Morphodynamic Signature of Tumor Invasion

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Solid evidences have shown that during tumor dissemination cancer cells modulate the morphodynamics of invasive protrusions to optimize their migration efficiency. However, it is yet unclear how the stochastic fluctuations of tumor-associated protrusions regulate the early invasion events in more complex multi-cellular tumors, and how they correlate with their metastatic potential. In this work, we used a reductionist model based on tumor micro-spheroids with increasing invasion capability to investigate the role of fluctuating protrusions in breast cancer progression [1]. To quantitate fluctuations, we defined a new set of key biophysical parameters that precisely correlated with the invasive potential of tumors. We showed that by perturbing protrusion activity using chemotherapeutics (Doxorubicin) and pharmacological inhibitors (C3 transferase) of key signaling pathways (Rho pathway), tumor invasiveness was significantly altered. Next, 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. Finally, to better investigate how tumor-associated protrusion fluctuations invade the tumor microenvironment and initiate metastasis, we have created a new organ-on-a-chip model of the human microcirculation embedding blood and lymphatic vasculature [2]. 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.

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SOP2

Hyaluronan of Low Molecular Weight Triggers the Invasive "Hummingbird" Phenotype on Gastric Cancer Cells

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Gastric cancer (GC) presents the highest mortality rate in the 21st century. [1] The GC occurrence and progression are associated to different causes namely, genetic bias, dietary habits, smoking, among others. However, there is a strong correlation between the infection by the bacteria *Helicobacter pylori* (*H. pylori*) and the GC incidence. The translocation of the CagA protein from the *H. pylori* to the host epithelial cells are associated to the most aggressive outcomes of GC. The composition of the extracellular matrix (ECM) is also related to the invasiveness of cancer cells, in particular the expression of the glycosaminoglycan Hyaluronan (HA). The dysregulated synthesis and degradation of HA in the tumour microenvironment leads to its accumulation in the cellular milieu with different molecular weights (Mw). Since HA is involved in diverse signalling pathways, its accumulation modulates pathological and physiological processes in an HA-dependent manner: short chains of HA (<100 kDa) are associated to cancer progression and metastasis, whereas long chains of HA are associated to cancer recession. [2] Considering these HA bioactivities and its capacity to modulate the behaviour of cancer cells, we developed a 2D platform to assess the impact of HA size on the formation of the invasive "*Hummingbird*" phenotype on AGS cells (derived from an epithelial GC). We build a layer-by-layer (LbL) platform through the sequential deposition of Poly-L-lysine (PLL) and HA of different Mw. This way it was possible to mimic the presence of HA in the physiological environment and study the impact of HA Mw on the migration and invasiveness of GC, in particular de activation of the invasive phenotype "*Hummingbird*". Our results demonstrate that a LbL constructwith HA of low Mw (*i.e.*, 5.6 kDa) induces increased polarity and morphometric changes on AGS cells resembling the "*Hummingbird*" morphology, with increased cellular motility and high expression of p-ERK1/2 and p-AKT, which is linked to cancer cells' invasiveness. The low expression of p-AKT and ANX-4 is observed when in presence of high Mw of HA (*i.e.*, 1450 kDa), which is associated to cancer cells latency.

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SOP3

Matrix Stiffness – The Manipulator of Cell Behaviour

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Tissues stiffen with age or pathophysiological conditions such as fibrosis, cardiovascular disease or cancer. The stiffness of the extracellular matrix (ECM) is one of the key regulators or "The Manipulator" of cellular behavior. The mechanical cue regulates the growth, phenotype, and even the migration of cells. Different cells respond differently to the cue. Therefore, during the development of biomaterials that recapitulate the physiological dynamics of the diseased niche or repair the tissue in situ — the mechanical cue of the ECM should be taken into consideration. Hence, a biomaterial library with different mechanical stiffness is generated by blending the biocompatible silk fibroin of silkworm Bombyx mori with the bacterial polysaccharide gellan gum in different ratios [1]. While silk fibroin mimics the collagen signature of the tissue ECM, the gellan gum resembles the glycosaminoglycan of the ECM. During the development, repair or disease, the cells remodel the surrounding ECM, leading to change in ECM microarchitecture (pore size – interconnectivity). This dynamic phenomenon of ECM is also incorporated along with the stiffness in this biomaterial library. The soft biomaterials of this library with larger pores probe the response of cancer cells towards the formation of solid tumor-like structure while the stiffer ones with relatively small pore sizes facilitate the migratory phenotype of cells and enhanced bone mineralization [2]. Interestingly, these mineralized tissues then act as target site for cancer cells (breast or lung) to metastasize. The implementation of this biomaterial library is not only to generate scientific knowledge about the disease progression such as cancer but also screen the therapeutic interventions that target the stiffening of diseased tissues.

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SOP4

Laser Ablation Triggers EMT Associated Translational Response in Melanoma Tumor Spheroids

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Nanosecond pulsed lasers have become widely adopted in the field of cancer therapy, namely to generate reactive oxygen species via plasma for the triggering of immunogenic cell death [1-2], to deliver biomolecules intracellularly via optoporation [3] or for tumor ablation [4]. Despite this range of applications, the full impact of nanosecond pulsed lasers on cellular mechanisms is not fully understood. Epithelial to mesenchymal transition (EMT) is a key process in embryogenesis and wound healing but its role in cancer is tied to progression, invasion and resistance through the action of players like the transforming growth factor beta (TGF-ß) family. Here we proceeded to evaluate the effects of nanosecond pulsed laser ablation in the expression of known EMT players in tumor spheroids. For this effect, spheroids comprising the human melanoma cell line VMM-15, human dermal fibroblasts(hDFbs) or a mixture of both where cultured for 7 days, at which time partial ablation of the spheroids using a nanosecond 355nm laser was performed. Ablated spheroids were collected either 3 hours or 3 days later. Morphology recovery was assessed using time-lapse microscopy. Gene and protein expression were analyzed by Real-Time PCR, Western Blot and immunohistochemistry. Ablated spheroids displayed variable angular openings of the wound surface, with greater angular openings being verified for spheroids comprising both tumor cells and hDFbs. Wound closure was faster for spheroids containing fibroblasts, with Picrosirius red staining showing higher collagen deposition where laser ablation had been performed. Analyzing the gene expression of the EMT mediator TGFß1 confirmed that laser ablation triggered an up-regulation of this gene across all conditions. In addition, increased gene expression for TGFß1R, COL1α1, PLOD2, COL3α1 and FN-EDA was also observed. When looking into the expression of key proteins such as smooth muscle actin and phosphorylated SMAD 2/3, a tendency for higher expression upon laser ablation was confirmed. Overall, the aforementioned results show that laser ablation triggers the upregulation of known EMT players in melanoma cell spheroids. Additionally, we have shown that the ablation recovery time is highly dependent on the presence of a fibroblast component within the spheroids which can be an effect of varying biomechanical cues due to differential extracellular matrix deposition.

ACKNOWLEDGEMENTS: FCT/MCTES through the grants SFRH/BD/119756/2016 and IF/00347/2015 and EU Horizon 2020 research and innovation programme under the ERC grant CapBed (805411).

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SOP5

Hydrogel Bioarchitectures for Modelling and Digitalizing 3D Cancer Dynamics Toward the Discovery of Functional Drug Thresholds

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The 3D architecture and mechanics of living tissues govern several underlying biological events. Therefore, their proper *in vitro* modeling is essential for recapitulating responses of interest, namely for cancer microenvironments [1]. Microfluidic-driven hydrogel manipulation provides a unique platform for engineering hydrogel fiber architectures *via*flow control and precursor manipulation [2]. We will demonstrate how this process can be leveraged to create complex multi-compartment hydrogel fibers, namely 3D cancer/basement membrane/stroma structures – melanoma-on-a-fiber. In these architectures, the recapitulation of cancer cell invasion and proliferation begins shortly after biofabrication, resembling the responses described for the earlier steps preceding metastasis *in vivo*. We will also present how the 3D compartments and multi-cellular composition directly affect the response of cancer cells to drugs and the extent to which the recapitulation of 3D complexity – namely how the presence of stromal components and a basement membrane alter the *in vitro* drug outcomes [3]. Added to proper 3D modeling, extracting relevant and quantitative data from cancer models is a considerable challenge. Taking advantage of the unique ability of hydrogels to interact with light [4], we leveraged microfluidic-spun hydrogel fibers towards the first living optical fibers. First, we will demonstrate how the unique composition of these fibers enables the 3D invasive proliferation of cancer cells to occur, leading to the formation of mature cancer fiberoids in the light-guiding fiber core. Then, we will present how light-cell interactions contribute to an optical snapshot that can inform on cancer metabolic activity and fluorescent marker expression, with or without anti-cancer drugs. Finally, we take advantage of this fast, non-invasive process to digitalize 3D cancer progression over time in the presence of varying drug concentrations, successfully discovering anti-proliferative inhibitory thresholds with unprecedented efficacy [5]. Overall, these microfluidic engineered hydrogel fibers present a widely adaptable and accessible tool for miniaturizing and modeling 3D cancer events in purely soft 3D environments. In addition, these constructs pave the way for plastic-free *in vitro* studies and open novel possibilities for digitalizing cancer drug susceptibility into quantifiable biomedical data toward precision health solutions.

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SOP6

Colloidal and Fibrous Thermo/Magnetic-Responsive Platforms to Deliver Curcumin as an Antineoplastic Agent

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To improve the efficiency of the antineoplastic drugs, a combinatorial strategy consisting of on-demand release of the drug and a physical trigger to induce the cell apoptosis have been considered. This research aimed to design and evaluate two colloidal and fibrous smart magnetic platforms to control the release of curcumin in a combination with magnetic hyperthermia. In the first phase, the colloidal nanocarriers with the core of SPION and thermo-sensitive shell of pluronic F127/F68 were synthesized and loaded with curcumin. The maximum drug release happened at lower critical solution temperature (LCST) of the polymeric shell (45˚C). However, *in-vitro* studies on osteosarcoma cell-line namely MG-63 showed that the optimum efficiency and synergistic effect of curcumin and hyperthermia happened at 41˚C. In the second phase, the synthesized nanocarriers were coated on the PCL fibers using silk fibroin as the paste. The release of curcumin from these two platforms was compared. Although the release concentration increased in the fibrous platform due to the homogenous distribution of nanocarriers, the sensitivity of the system to the temperature decreased. To verify this effect, the stepwise release of the drug was studied in the frequent temperatures of 37 and 47 ℃. The main reason for this phenomenon could be the low temperature-dependency of the diffusion coefficient in silk fibroin comparing to pluronic. *In-vitro* studies showed that healthy hMSCs are less sensitive to curcumin/temperature combination comparing to MG-63 cell-line. The strategy proposed here could be used as a potential solution to replace the traditional cancer chemotherapy.

SOP7

Novel Surface- and Biochemistry Platforms for Cancer Biosensing, Therapy and Beyond.

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Many biotechnologies, primarily chemical detection and disease therapy, require intimate contact between a (bio)material or substrate with a biomolecular receptor. The majority of biomolecule immobilization methods presented in the literature fail to account for the structure, function, orientation and immobilization sites of the bioreceptor in question. Many relevant bioreceptors are large, complex, and often membrane-bound proteins or biomolecules. Therefore, to ensure optimal function of the biomolecule and biotechnology in general, careful strategies must be employed in order to retain biomolecule structure, which then translates into proper biomolecule function. It is in this context that the development of novel chemical platforms for the modification, functionalization and integration of (bio)materials developed here at I3Bs will be discussed:

1) Melanins are natural biological pigments found in most animal species. Melanin nanoparticles have shown promise in theranostic application due to among other properties, their ability to be excited by near-infrared irradiation. A property which allows melanins to be utilized for simultaneous photoacoustic imaging and photothermal therapy. We have developed a surface chemistry platform for the modification of melanins and applied them to eumelanin nanoparticles, purified from cuttlefish. The platform consists of the silane grafting of (3-Glycidyloxypropyl) trimethoxysilane (GPTMS) to facilitate site-specific and regioselective functionalization of eumelanin nanoparticles; which have been evaluated with small molecules, peptides, and antibodies. This platform allows for the facile functionalization of the eumelanin nanoparticles which can then be utilized as a specific tissue targeting (e.g. a tumor) theranostic for simultaneous photoacoustic imaging and photothermal therapy.

2) Aptamers are synthetic nucleic acid molecules, primarily DNA or RNA, selected through an evolutionary selection process to bind specific molecular targets that range from small molecules to other biomolecules to whole cells referred to as nucleic acid antibodies. We aim to incorporate aptamers that feature different binding mechanisms into nanoporous layer-by-layer formed polyelectrolyte films. Aptamer ligand diffusion into the nanoporus film would trigger aptamer binding and film destruction. Such films could serve as the biorecognition element of a surface-sensitive biosensing platform or be formed into particles for environmentally responsive drug delivery.

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SOP8

O-Glycotripeptides as Minimalistic Molecular Models of Glycoproteins

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Proteins are critical for cellular functions. To acquire functional activity, they must fold into defined three-dimensional structures known as native state. [1] Protein folding is a tightly controlled process but at some pathological conditions misfolding can occur that lead to formation of off-pathway aggregates. One of the physiological mechanisms to control the folding and to correct misfolding is the protein glycosylation: O-glycosylation is a post-translational modification of proteins that can alter the structure, properties and bioactivitiy of the modified proteins. [2] The exact molecular mechanism(s), the kinetics and the thermodynamics of these changes induced by glycosylation are still poorly understood. Herein, we propose a minimalistic model that uses tripeptides and their O-glycosylated analogues to study these processes. Such models are simpler in composition but can code specific bioinformation and transfer it to the studied system. [4] The molecular design is based on aggregation-prone tripeptides that contain either serine (S) or threonine (T) in the middle of the sequence. In the minimalistic glycotripeptides, glucose (Glc) is bound to S or T, thus copycatting the native O-glycosylation motif in epidermal growth factor-like (EGF) repeats found on numerous proteins including Notch. Using these models, we showed a distinct role of S, T and Glc in the glycopeptides stereochemistry and aggregation. We demonstrate that O-glycosylation at S differs from the glycosylation at T and affect the aggregation: we observed aggregates with different morphology, size, and mechanical properties. In addition, O-glycosylation affected the thermal stability of the formed assemblies, by increasing their melting temperature. These changes were more pronounced for S analogues as compared with T ones. Using computational modeling, we demonstrate that the differences between S and T analogues are due to distinct CH-pi interactions. These results show that the designed minimalistic O-glycotripeptides are promising tools towards better understanding of the glycosylation role in different physiological and pathological processes and for design of new functional biomaterials.

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SOP9

Silk Fibroin-based 3D *In Vitro* Breast Cancer Model for Drug Screening Applications

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Breast cancer is amongst the most diagnosed types of tumor in women. Treatment response can vary in different tumor subtypes, with numerous patients developing resistance to standard therapies [1]. In recent years, significant efforts have been made to generate 3D *in vitro* tumor models, to discover novel potential treatments against cancer development and progression [2-3]. In this work, enzymatically-crosslinked silk fibroin (eSF) hydrogels are used to assemble a 3D breast cancer *in vitro* model (3D-BCM). For 3D-BCM generation, breast cancer cells (MCF7 cell line) and mammary fibroblasts were encapsulated into eSF hydrogels, and their behavior was evaluated up to 14 days of culture. The results showed eSF hydrogels stiffness increase during time, mimicking spontaneous tumor stiffening. As compared to MCF7 monoculture, 3D-BCM co-cultured cells exhibited an augmented formation of spheroid-like clusters already in the early days of testing. Moreover, co-cultured cells showed an upregulated gene expression, specifically of genes involved with the remodeling of the extracellular matrix, and with fibroblasts switch into the cancer-associated type. In addition, the developed 3D-BCMs showed differential response to the treatment with doxorubicin and paclitaxel, conventional chemotherapeutics, when cells were cultured in single or co-culture conditions. Our results indicate that eSF hydrogels are suitable matrices to support a 3D breast cancer *in vitro* model development. This model could be further used to unravel new molecular targets for anti-cancer therapeutics.

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SOP10

A New Chalcone Derivative for Glioblastoma Treatment

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Glioblastoma (GBM) is the most common and most deadly primary malignant brain tumor. Current therapies have limited efficacy, being the average survival of GBM patients after diagnosis limited to a few months. The drug resistance ability of the tumor and fast regrowth of GBM are some of the problems related to these treatments. Moreover, the intrinsic high heterogeneity of these tumors is one of the main reasons for the low efficacy of the available treatments. Therefore, the discovery of new treatments with enhanced efficacy for this highly aggressive brain cancer is urgently needed. Chalcones are synthetic and naturally occurring compounds that have been widely investigated as anticancer agents. In this work, chalcone derivatives were tested regarding their inhibitory activity and selectivity towards GBM cell lines. A specific chalcone derivative shown to have potent and selective cytotoxic effects towards GBM cell lines, being further investigated regarding its ability to reduce critical hallmark features of GBM. This derivative shown to successfully reduce the invasion and proliferation capacity of tumor cells, both key targets for cancer treatment, by inducing cell cycling arrest and cell apoptosis. Moreover, to overcome potential systemic side effects and its poor water solubility, this compound was successfully encapsulated into liposomes. Therapeutic concentrations were incorporated retaining the potent *in vitro* growth inhibitory effect of the selected compound. In conclusion, our results demonstrated that this new formulation can be a promising starting point for the discovery of new and more effective drug treatments for GBM.

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SOP11

Gastrointestinal Organoids-on-Chip: Challenges and Future Trends

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The gastrointestinal system has been the subject of focus of scientific research for many years, due to its essential role in the individual well-being. Moreover, its role in physiological aspects such as metabolism and digestion, and its evident connection to the immune system and recently the microbiota-gut-brain axis, makes it worth investigating under several aspects [1]. The gastrointestinal system comprises the mouth, pharynx, esophagus, stomach, small intestine, large intestine, rectum, anus, liver and pancreas. Given the relevance of such an important group of organs, related homeostasis and diseases have been widely studied. Several types of disorders, from cancer to inflammatory diseases affect this group of essential organs, making it essential to further advance the medical know how and personalized therapies. The present challenge affecting the drug development pipeline relies in the accomplishment of high predictability in drug treatment, ensuring minimal unanticipated lateral effects. Consequently, translational research must rely in complex models mimicking human conditions instead of depending on 2D, 3D static and animal models. One of these models are organoids-on-chips. Important discoveries have paved the way, namely the development of organoids, which have now been widely utilized for studying both elementary and medical biology due to complex and reliable *in vivo* mimicking of organoids [2]. Organoids-on-chips are organoids cultured in microfluidic systems, recaping important features such as the 3D structure, cell-cell and cell-materials interplay, present in pathology and normal human organs *in vitro*. Furthermore, integrated multiple organs on one chip are progressively more sophisticated in terms of ADME and toxicity demonstration, and used for a better understanding in drug interaction and fate, promising unlimited potential in predicting drug efficacy and safety. Advances in terms of gastrointestinal organoids-on-a-chip models are vast, seizing the opportunity to the next step into the clinical trials.

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SOP12

Marine-based Nanaoparticles with ERBB-2 Antibody Immobilized Target Breast Cancer Cells Both *in vitro* and *in vivo*

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An effective anti-cancer therapy aims to eliminate tumor cells without toxic effects on the surrounding healthy tissues. Since current chemotherapeutic drugs may present severe side effects, great efforts have been made in the development of alternative and well-tolerated therapies. The targeting of specific receptors present in cancer cells is an interesting approach to develop successful cancer treatments. Since ErbB-2 (human epidermal growth factor receptor 2) is overexpressed in around 30% of breast cancers, the respective antibody was immobilized at the surface of marine-polymeric nanoparticles (NPs). Fucoidan/chitosan NPs incorporating gemcitabine (NPs+Gem) were produced by polyelectrolyte complexation as previously optimized by our group, and their surface functionalized with ErbB-2 antibody (NPs+Gem+Ab) [1]. The maximum antibody immobilization was optimized as 10 µg mL-1. NPs+Gem+Ab present size of 159±23 nm, a polydispersive index of 0.178±0.027, and a zeta-potential of 21.30±2.68 mV, indicating that nanoparticles are monodisperse and stable. The targeting ability of NPs with immobilized ErbB-2 antibody was validated by the increased cellular uptake of SKBR3 cells (ErbB-2 positive breast cancer cells) as compared to MDA-MB-231 (ErbB-2 negative breast cancer cells). To further validate the efficacy of the targeting, a co-culture system was established with human endothelial (EA.hy.926) and breast cancer cells (SKBR3). Similar cytotoxicity was observed for Gem, NPs+Gem, and NPs+Gem+Ab concerning the effects on endothelial cells after 24h (between 24-30%). Additionally, NPs+Gem+Ab presented toxicity to breast cancer cells around 80%, whereas free Gem and NPs+Gem only presented toxicity around 12% and 23%, respectively, confirming the efficacy of the targeting system. In an *in vivo* study, where SKBR3 cells were injected into the mammary fat pad of immunocompromised mice, NPs+Gem+Ab induced tumor growth impairment. Furthermore, tumorigenic mice treated with NPs+Gem+Ab presented less metastatic lungs. The targeting efficacy of the NPs+Gem+Ab system was validated both *in vitro* and *in vivo*, being the developed system a possible approach for ErbB-2 positive breast cancer treatment.

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SOP13

Melanin Nanoparticles as a Theranostic Approach for Cancer Treatment

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By targeting specific cells without destroying the healthy environment, a more effective treatment method than traditional therapies can be obtained. Controlled and targeted drug delivery has become one of the main topics of current scientific research. In this context, "smart materials" have the ability of modulating the drug release profile under specific stimuli, at a specific time and target site. Inherently conductive polymers (ICPs) present electrical, magnetic and optical properties and when coupled to a drug can be a powerful and facile system since, we hypothesize, that the drug release profile can be controlled by applying an external electrical current as a stimulus, mediated through alterations in the redox state of ICPs.[1] Melanin is a natural biodegradable and biocompatible material, that has shown great advances in different research fields such as biomedicine, nanotechnology and bioengineering. Therefore, due to its inherent properties, melanin nanoparticles show great promise in the development of new controlled delivery systems and new therapies based on changes in internal or external conditions. NPs are more selective and specific to a tumor site, allowing an optimization of the dose, diminishing drug side effects, which is the main problem associated with anticancer drugs administration.[2] Photothermal therapy (PTT) has emerged as a minimal invasive approach for the destruction of cancer cells without damaging the surrounding healthy tissue. This strategy is possible due to the absorbance of energy by the MNPs, induced by near infrared light (NIR), allowing MNPs to release a great amount of vibrational energy, which turns into heat. The possibility to induce heating by NIR light irradiation, in the specific site where tumor is located, enhances the targeting effect and efficacy of the therapeutics. [3] Herein, the focus is to develop a drug delivery system to target cancer cells that presents biocompatible and noncytotoxic features, but one that can be capable of cancer cell destruction without damaging the surrounding, healthy, tissue. Therefore, MNPs were functionalized with polydopamine and polypyrrole to precisely control the release of dexamethasone (Dex) and the photothermal effect of MNPs was assessed.

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NOTES

ABSTRACTS LIST

P01

DEVELOPMENT OF HIERARCHICAL SCAFFOLDS FOR BONE TISSUE ENGINEERING APPLICATIONS

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The bone tissue engineering field has been pursuing new strategies to recreate bone matrix structure, composition, and hierarchy at different length scales. In this reasoning, the present study's main aim was to develop an advanced hierarchical scaffold capable of mimicking the structure, mechanical properties, and composition of bone tissue to some extent. For that, silk fibroin was combined with decellularized cell-derived extracellular matrix and reinforced with carbon nanotubes. The scaffolds were prepared using enzymatic cross-linking, freeze modeling, and decellularization methods. First, their structure and mechanical properties were assessed, showing that the developed scaffolds were elastic and with pore sizes (≈ 112 ± 22 μm), total porosity (75 ± 3%), and stiffness's (≈ 5 kPa) within the range described for the stimulation of cell's differentiation along the osteogenic lineage. Then, the bioactivity *in vitro* of the developed scaffolds was investigated, showing the formation of mineralization. The cellular *in vitro* studies using human adipose-derived stem cells (hASCs) demonstrated that scaffolds supported cell adhesion, spreading, proliferation, and ultimately, osteogenic differentiation. The positive influence on osteogenic differentiation was confirmed by collagen secretion, increased ALP activity, and expression of osteogenic-related genes (e.g., ALP, Runx-2, Col Iα, and OPN). Furthermore, the histological stainings showed cells infiltration into the scaffolds, and the hemolytic assay established the hemocompatibility of the hierarchical scaffolds. The promising results showed that the developed carbon nanotubes-reinforced cell-derived matrix-silk fibroin hierarchical scaffolds could be used for bone tissue engineering scaffolding applications.

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P02

ENGINEERING SILK FIBROIN/CHOLINIUM GALLATE BASED SPONGES ENVISIONING INFLAMMATORY DISEASES TREATMENT

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The use of natural polymers to engineer 3D-based matrices as therapeutic solutions to tissue regeneration has been the focus of intense research. Functional architectures made from biocompatible materials can be designed with specific activities to meet the needs to treat diseases[1]. Herein, a green approach was developed combining silk fibroin (SF), a natural protein, with Ch[Gallate], a phenolic-based biocompatible ionic liquid with antioxidant and anti-inflammatory features. 3D-based sponges were produced through the dispersion of the Bio-IL (up to 3% w/V) into SF aqueous solution, followed by freeze-drying. Firstly, the Bio-IL was synthesized, and its synthesis was validated by ^{1}HNMR. The antioxidant potential of the produce Bio-IL and its precursor, gallic acid and the SF/ Ch[Gallate] based sponges, was assessed using the DPPH assay. The antioxidant activity of the samples was maintained up to 24h either in the precursor and the Bio-IL, Ch[Gallate], as well as in the produced sponges The changes in the secondary structure of the SF/Ch[Gallate]-based sponges studied by FTIR revealed that the Bio-IL addition increased its β-sheet content (up to 65%), compared with SF sponges produced without Bio-IL (45%). The morphological analysis of the matrices was performed by micro-CT, revealing porosity percentages up to 76.6 %, and pore sizes up to 70 µm. The SF/ Ch[Gallate]-based sponges showed high water uptake ability up to 1900%, after 7 days, and the Ch[Gallate] release profile was controlled over time. Human adipose stem cells were cultured into the structures for up to 7 days, and no deleterious effect on cellular metabolic activity, morphology, or proliferation was observed. Moreover, the therapeutic effect of the sponges was studied by quantifying the tumor necrosis factor(TNF-α) in differentiated and stimulated THP-1 cells up to 7 days, and dexamethasone was used as a control. The findings revealed that the matrices decreased the TNF-α release throughout the culture time, suggesting that those biomaterials would provide a prolonged effect compared to the free drug. Overall, physicochemical and biological features of the structures can be designed by modulating the Bio-IL content. Therefore, the obtained results suggested that the SF/Ch[Gallate]-based sponges have the potential to be used as tissue engineering solutions, targeting diseases with associated inflammatory processes.

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P03

DIFFERENT APPROACHES TOWARDS EFFECTIVE CURCUMIN-BASED ANTIMICROBIAL PHOTOTHERAPY

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Nowadays, microbial-related diseases are a relevant health concern at a global scale mainly due to the emergence of antimicrobial resistance (AMR). For instance, bacteria can adhere to a surface and become enclosed in matrix of extracellular polymeric substances forming a structure known as biofilm. Typically, while in this state bacteria act as a well-organized community showing greater resilience against several antimicrobial agents. In this context, light-based therapies such as photodynamic therapy (PDT) have shown great promise. PDT can be summarized as an oxygen-dependent photochemical reaction where the activation of a photosensitizer, upon exposure to light, leads to the generation of reactive oxygen species (ROS) resulting in a cytotoxic effect. Despite being a promising approach PDT is plagued by some issues namely, low selectivity and uptake into microbial cells as well as insolubility in aqueous media of many photosensitizing agents. Curcumin (Cur), a phenolic compound with remarkable biological properties, has been explored as a possible alternative to classical photosensitizing agents due to being blue-light responsive, which is better tolerated by human cells than the typically applied UV radiation. Nevertheless, Cur presents solubility issues in aqueous media and is unstable at physiological pH. To tackle these issues, this study aimed to compare the efficacy of methyl-ß-cyclodextrin, and a deep eutectic solvent (Des) based on glycerol (Gly) and choline chloride (ChCl) as Cur vehicles for antimicrobial PDT. The obtained results showed that while both vehicles are effective solubilizers of Cur, Des displayed the highest solubilization capability. Posteriorly, the antioxidant properties were assessed with the Des-based Cur formulation showing greater antioxidant potential over a 14-day period. The photodegradation of Cur in the formulations when exposed to blue-light was also assessed, with the cyclodextrin-based formulation displaying a significant photoprotective effect, while Cur in Des displayed behaviour similar to standard solvents. Regarding, cytocompatibility with L929 cells, basal antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* as well as photo-induced toxicity, the Des-based Cur formulation was superior in all accounts. Overall, the results showed that the Des-based Cur formulation possessed greater phototherapeutic potential and hence it will serve as basis for on-going studies aiming to develop novel phototherapeutic approaches tackling the problematic of established biofilms.

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P04

SHARK COLLAGEN AND CHONDROITIN SULFATE HYDROGELS ENVISAGING CARTILAGE TISSUE ENGINEERING

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Marine species are extraordinary reservoirs for extracting biomolecules, and their uniqueness provides an excellent opportunity for the application of valuable biological materials in different areas [1]. Astoundingly, shark skeletons constituted by tesseral architecture combined different types of cartilage such as areolar calcified cartilage, a highly cellular structure typical present in the vertebral centra, a calcified cartilage tissue consisting of spherules of hydroxyapatite, and prismatic calcified cartilage, a densely mineralized tissue associated with the outermost region of perichondral tesserae, interconnected by collagenous fibers and also unmineralized, hyaline-like cartilage [2-4]. Shark-derived materials have received enormous attention regarding a possible application for articular pathologies, being also currently explored for the extraction of collagen (COL) protein, chondroitin sulfate (CS) glycosaminoglycan, and production of marine biomaterials with application in biomedicine [5, 6]. In this study, shark materials derived from *Prionace glauca' cartilage byproducts* obtained in Portuguese fish processing plants were used as raw materials for the development of biomaterials. The aim is to fabricate stable shark-derived hydrogels by combining marine collagen with chondroitin sulfate to mimic the human cartilage extracellular matrix, making it a suitable biomaterial for anchoring the chondrogenic cell line. Swelling test and rheological mechanical properties demonstrated a stable polymeric matrix in aqueous media. Moreover, *in vitro* biological performance is being evaluated by culturing ATDC5 cells and further addressing cell adhesion, morphology, and distribution, by fluorescence microscopy following Phalloidin/DAPI staining, while cell metabolic activity will be measured by MTS assay, envisaging the engineering of new cartilage tissue.

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P05

ACEMANNAN-BASED TERNARY BLENDED FILMS: A NATURAL APPROACH ENVISIONING BIOMEDICAL PURPOSES

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The scientific community is propelling the exploitation of natural origin macromolecules, as complex physical mixtures or drugs, to be applied in biomedical or tissue engineering (TE) solutions. Aloe Vera (AV) is a highly explored medicinal plant, from which the main polysaccharide is acemannan (ACE)[1-3]. The combination of ACE with chitosan and alginate results in established interactions that lead to mixed junction zones formation, predicting improvement of membrane functionality[4,5]. This work proposes the development and physicochemical characterization of ACE-based blended films as a promising strategy to design a nature-derived bioactive platform. For that purpose, solutions based on ACE, ALG and CHT were prepared using water and acetic acid 1%(v/v), respectively. The blended solutions were optimized using previously defined volumes of the original solutions. Those were casted in the moulds and left to dry at 37ºC for 24h. The films were further characterized using different techniques to define their physical features and chemical composition, as well as the interaction established between the matrices into the blended formulations The results confirmed that more resistant and stable complex polyelectrolytes structures were formed, through electrostatic interaction, divalent gelation, and hydrophilic-hydrophobic interaction. Furthermore, the studied compositions present good dimensional stability (G'>G'') and ductile fracture behavior. They also show good flexibility, and adequate swelling ability with a mostly radial water uptake and a sustainable ACE release to the medium, being favored at intermediate acidic pH of 4.6. A comparative observation between the ternary compositions reveals that the 4:3:1 formulation presents a lower ACE release to the media at pH 4.6 and 7.0, which is in accordance with the enhanced mechanical behavior of 4:3:1 composition that suggests the existence of higher network stability due to strongest chemical interactions between the polymeric chains. Moreover, the non-cytotoxic profile of ACE-based films and the positive biological performance (cell attachment, spreading, and viability) when using fibroblasts suggest that they can be a promising in TE strategies, preferably with potential to act as bioactive topical platforms.

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P06

UNCONVENTIONAL SECRETION OF RHAMM BY BREAST CANCER CELLS

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The substantial majority of deaths associated with breast cancer results from metastization, and therefore, the ability of cancer cells to spread and colonize to different locations represents a critical stage in the pathogenicity of a tumor. Secretion of pro-invasive and pro-metastatic proteins is an important mechanism in cancer cells signaling that can promote this behavior. Typically, secreted proteins containing a signal peptide are targeted to the endoplasmic reticulum (ER)-to-Golgi route for proper folding and packaging, but both normal and cancer cells can also use unconventional pathways (that bypass the classic ER-to-Golgi route) to secrete proteins including pro-invasive factors. An example is the receptor for hyaluronan mediated motility (RHAMM, encoded by the HMMR gene and also designated CD168), an oncogenic cytoplasmic protein with extracellular functions. Although RHAMM lacks a conventional signal peptide, it can be found at the cell surface as a complex with other proteins[1]. Gathered evidences indicate that RHAMM follows unconventional protein secretion (UPS) pathways to the cell surface[2], but the mechanisms underlying this UPS are largely unknown. From the four types of UPS, only three involve cytoplasm soluble proteins: non-vesicular UPS through membrane pores (I) or via the ABC transporters (II) and vesicular UPS through autophagy-associated vesicles(III). Bioinformatic analysis of RHAMM sequences identified putative LC3-interacting regions that could account for the interaction with vesicles and consequently secretion via autophagy pathways[3]. Because HMMR is a breast susceptibility gene, we have selected breast cancer cell lines with different expression levels of RHAMM: MDA-MB-231, Sk-Br3, and HS578T. MCF10A - a normal breast cell line was used as a control. The expression of RHAMM in these breast cancer cells was characterized by Western blotting and immunocytochemistry in the absence and presence of autophagy inhibitors/inducer (chloroquine and bafilomycin A1, metformin). Western blotting data showed the presence of several truncated forms of RHAMM that can be generated either by alternative splicing or enzymatic cleavage. RHAMM was also detected in the culture medium. The RHAMM protein profile changed in the presence of autophagy inhibitors, as well as its intracellular expression levels in the tested cell lines, suggesting an involvement of autophagy pathways in the distribution of RHAMM in the cell. References 1.Carvalho, A.M., et al., Acta Biomater,2021. 119:p114-124. 2.Maxwell, C.A., et al., J Cell Sci,2008. 121:p925-32. 3.Cruz-Garcia, D., et al., bioRxiv,2017:p122028.

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P07

BIOACTIVITY SCREENING OF CYANOBACTERIA FOR IDENTIFICATION OF NOVEL ANTICANCER COMPOUNDS

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Cancer is a leading cause of death worldwide, responsible for an estimated 10 million deaths in 2020 [1]. Moreover, the number of cancer-related deaths is expected to increase to 16.4 million by 2040 and, therefore, there is a clear and urgent need for novel drugs with increased efficacy for the treatment of different cancers. In the last decades, a significant number of novel compounds with potent pharmacological properties has been discovered from the marine organisms [2]. Cyanobacteria have gained significant importance due to their ability to produce secondary metabolites with biological activity, useful in the treatment of various diseases, including cancer. This work aimed to assess the anticancer potential of marine cyanobacteria isolated from the Portuguese coast. For that, the cytotoxicity of different fractions with increasing polarity, obtained from multiple strains of the LEGE Cyanobacteria Culture Collection, was assessed by the MTS assay in two tumor cell lines: MDA-MB-231 (human breast cancer) and A549 (human lung cancer). Some fractions significantly decreased the metabolic activity of both tumor cell lines and the highest percentage of inhibition was observed in fractions LEGE04288 B and C, and LEGE06155B, which suggests the presence of cytotoxic compounds. These strains were submitted to another process of fractionation and fractions LEGE04288 E and F, and LEGE06155 B, G and H remarkably decreased the metabolic activity of MDA-MB-231 and A549 cells. Indeed, LEGE06155 G completely abolished the metabolic activity of both tumor cell lines. This behavior was attributed to the presence of bartolosides produced by the strain LEGE06155, with further tests showing that bartoloside B completely abolished the metabolic activity of both tumor cell lines.

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P08

INVESTIGATING THE ROLE OF CELL ADHESIVE CUES IN TUMOR PROGRESSION USING GELLAN GUM-BASED TISSUE ENGINEERED 3D OSTEOSARCOMA MODEL

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The complexity and heterogeneity of cancer results in the lack of effective treatments to tackle its different forms. Osteosarcoma is a rare cancer type. Nonetheless, it is the most frequent primary malignancy in bone[1], and the poor prognosis of metastatic disease increases the need for more efficient therapies[2]. Therefore, the development of a more representative tissue engineered 3D osteosarcoma model would help to unwind new therapeutic targets and better predict the clinical outcome. Matrigel is considered as "golden standard" scaffolding material for 3D cancer modeling; however, it has its limitations[3]. Hence, we are developing a 3D tumor-engineered osteosarcoma model using a modified gellan gum-based hydrogel. Although the tumor microenvironment regulates the behavior of osteosarcoma cells in countless ways, our model focuses on investigating the role of cell adhesive cues on disease progression. Thus, gellan-gum is functionalized with divinyl sulfone[4] to incorporate different cell-adhesive peptides including CGRGDSP (integrin-binding), CKRSR[5] and CFHRRIKA[6] (heparan sulfate-mediated). This resulted in enhanced viability of osteosarcoma cells within the hydrogels. The investigation of the effect of adhesive cues on cell morphology and gene expression is ongoing. We hope this model helps expand our knowledge in the way osteosarcoma cells interact with the microenvironment and in designing therapeutics targeting the cell-matrix adhesion.

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P09

OSTEOGENIC LITHIUM-DOPED BRUSHITE CEMENTS FOR BONE REGENERATION

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Brushite cements are recognized for excellent osteoconductivity and rapid resorption rate, with improved bone regeneration capacity compared to hydroxyapatite-forming cements. Doping of biologically relevant ions in brushite has been performed to increase the mechanical and biological performance of bone substitutes.[1] Particularly, lithium (Li^+) is a promising trace element to encourage the migration and proliferation of adipose-derived stem cells (hASCs) and the osteogenic differentiation-related gene expression, important for the osteogenesis. This study aims to investigate the osteogenic performance of new brushite cements obtained from Li^+-doped ß-tricalcium phosphate as a promising strategy for bone regeneration. The cements containing 5 mol.% Li^+ were prepared with 3 wt.% phytic acid as setting retarder using liquid-to-powder ratio of 0.3 mL/g.[2] *In-situ* X-ray diffraction and in-situ 1H-nuclear magnetic resonance measurements proved the precipitation of brushite and monetite, indicating that Li^+ favoured the formation of monetite under certain conditions. Li^+ was detected in the remaining pore solution in significant amounts after completion of hydration. Isothermal calorimetry results showed an accelerating effect of Li^+ with increasing setting retarder concentration. A decrease of initial and final setting times with increasing amount of Li^+ and setting times could be well adjusted by means of varying the setting retarder concentration. *In vitro* assays using hASCs cultured on the powdered cements showed normal metabolic and proliferative levels. The immunodetection and gene expression profile of osteogenic-related markers highlights the incorporation of Li^+ for increasing *the in vivo* bone density. Overall, the tunable properties of the developed Li^+-doped brushite cements and its osteogenic potential evidenced by the significant up-regulation of Col Iα gene expression and ALP, show the promising benefits of these materials for bone regeneration, namely for filling bone defects.

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P10

NANOPATTERNED AND PEDOT-CONTAINING SILK FIBROIN NERVE GUIDANCE CONDUITS FOR PERIPHERAL NERVE REPAIR

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Injuries of peripheral nervous system are a major source of disabilities; the mobility of muscles gets impaired, sensations suffer distortions and can lead to painful neuropathies.[1] There is a critical nerve gap of around 5 mm length beyond which nerve regeneration does not happen. The progress in tissue engineering has resulted in the development of artificial nerve constructs that can guide and facilitate the axonal nerve growth.[2] The present work shows the synthesis and physico-chemical characterization of NGCs made mixing Silk Fibroin (SF) and poly(3,4-ethylenedioxythiophene) (PEDOT) nanoparticles at 1, 5 and 10 %. Fast restoration of the electrophysiological stimulatory capacity will be key for functional recovery. PEDOT is an electroconductive polymer which would allow to avoid muscle denervation while the nerve gap gets regenerated. Also, conduits are synthesized with an inner pattern which would allow the guiding of neural regeneration in the longitudinal axis. Mechanical properties have been demonstrated to be a highly important regulator of axonal regeneration and elongation.[3] Results have shown conduits with a Young's modulus and reaction to tensile loads in the range of nerves. Also, they are flexible and do not occlude, which is highly important as they may be placed in flexible body parts. Conduits are tested as supports for SCs proliferation and BJ fibroblasts cannot infiltrate through the walls, which can be advantageous for avoiding the undesired *in vivo* fibrotic tissue formation in the lumen.

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P11

OPTIMIZATION OF THE DECELLULARIZATION OF FEEDER LAYERS AS A SUBSTRATE FOR THYMIC CELL CULTURE

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Thymic epithelial cells (TECs) are key players on T cell development. Since TEC cultures are difficult to establish *in vitro*, feeder layers were used as platforms for successful TEC culture. To overcome the contamination issues of the use of feeder layers, it is relevant to explore the potential of feeder layer-derived extracellular matrix (ECM) on TEC cultures. Such studies can be performed in flat compact substrates. However, those do not facilitate the development of multilayer feeder cell cultures. Due to the high surface area and porosity, electrospun fibrous meshes (eFMs) can used as a support for ECM deposition. Therefore, the aim of this work was to assess the effect of candidate decellularization protocols (Triton/ammonium hydroxide based and SDS-based) and feeder layer cell seeding densities on TEC culture. Firstly, the ECM of feeder layers was efficiently recovered after both decellularization protocols, maintaining ECM proteins. The decellularized ECM was characterized and it was constituted by fibronectin, laminin, collagen type I and type IV. The 0.1M seeding density revealed a higher amount of most ECM proteins, with the exception of fibronectin. All the decellularized matrices allowed the diffusion of FITC-Dextran 20kDa, however at a slower pace than bare eFMs. TEC cultures confirmed that the ECM amount impacts on cellular performance, with higher densities showing a decreased activity overall over decellularized matrices. From the tested decellularization protocols, the Triton/ammonium hydroxide based one at a cell density of 0.05M is the most promising to be applied on thymic bioengineering strategies.

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P12

GALLIC ACID-BASED COMPOUNDS AS MODULATORS OF THE SUPRAMOLECULAR ASSEMBLY OF AMYLOID β PEPTIDE IN ALZHEIMER DISEASE

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Polyphenols present a variety of biofunctional activities that are relevant for the biomedical field, *e.g.* anti-microbial, anti-oxidant or anti-amyloidogenic.[1] In the context of neurodegenerative disorders, *e.g.*, Parkinson's or Alzheimer's disease (AD), these molecules can act as modulators of the supramolecular assembly and deposition of toxic proteins/peptides, that are hallmarks of these diseases, *e.g.*, amyloid b (Aβ42) in the case of AD.[2] Importantly, it has been reported that the bioactivity of polyphenols is based on the number of gallic acid (Ga) units in their chemical structure, *e.g.*, epigallocatechin gallate presents two units in its structure.[3] Herein, we obtained two cork Ga-based extracts (vescalagin and castalagin presenting five Ga units), and we synthesized three dendrimer-based polyphenols' mimics, namely 2G0-GaOH, 2G1-GaOH and 3G1-GaOH, exhibiting two, six and nine Ga units, respectively. We evaluated the ability of the cork's polyphenols and Ga-terminated dendrimers to interact with Aβ42 and remodel its supramolecular assembly into non-cytotoxic conformations. Our results show that the number of Ga units (and their molecular presentation) influence the capacity of these molecules to modulate the Aβ42 fibrillization. In fact, through several techniques such as WB, CD, Thioflavin T, STEM or AFM, we found that vescalagin and the synthetic compounds, 2G1-GaOH and 3G1-GaOH (presenting higher number of Ga units per dendrimer) were more efficient at reducing the amount of Aβ42 cytotoxic forms. In the cell studies, they were also able to remodel the supramolecular assembly process of Aβ42 and rescue cells from the Aβ42 monomers/oligomer toxicity, showing that the multivalent presentation of Ga units and their spatial presentation are important parameters that are able to enhance their interaction with Aβ42. Overall, Ga-based compounds can be further exploited as anti-amyloidogenic molecules able to modulate the pathological assembly of peptides/proteins in neurodegenerative diseases.

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P13

STEM CELL-BASED ADVANCED THERAPY FOR NEURODEGENERATIVE DISEASES

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Neurodegenerative diseases (NDs; e.g. Alzheimer's disease, Parkinson's disease, multiple sclerosis -MS, and Huntington's disease) result from progressive structural and functional degeneration of particular subsets of neurons within the central nervous system (CNS) [1]. Currently, they have no effective treatment, but advanced therapeutic medicinal products (ATMPs), and, particularly, mesenchymal stem cells (MSCs) emerged with the potential to cure NDs. However, despite high initial expectations, their clinical use is still limited. To overcome their crucial limitations, such as poor cell survival and low penetration into the CNS, we designed a hydrogel to deliver bone marrow (B)MSCs intrathecally or intracerebroventricularly. The hydrogel is based on biomolecules naturally present in the CNS and physically crosslinked with liposomes, presenting also the ability to carry other therapeutic agents. After hydrogel physicochemical characterization, its biocompatibility was confirmed *in vitro* with BMSCs and *in vivo* through its intracerebroventricular injection in rats. Moreover, the distribution of the engineered hydrogel into corpus callosum can be ideal for NDs treatment since the damage of this white matter structure is responsible for important neuronal deficits [2]. To assess its efficacy, we selected MS, the main cause of chronic neurologic disability in young adults. Therefore, the efficacy of the developed formulation containing a lower number of cells than previously reported [3] was demonstrated using an experimental autoimmune encephalomyelitis (EAE) rat model. The BMSCs-laden hydrogel significantly decreased the maximum mean clinical score and average mean clinical score when compared with the control group of EAE and eliminated the relapse. Therefore, this strategy may bring a new hope for MS and related debilitating conditions patients.

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INFLUENCE OF THERMAL CONDITIONS IN THE 3D MICROARCHITECTURE OF THE SPONGY-LIKE HYDROGELS

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Gellan gum (GG) spongy-like hydrogels (SLH) have been widely explored for several tissue engineering applications due to their attractive properties such as mechanical stability and cell-adhesion features. Those are prepared from GG hydrogels after consecutive steps of freezing and freeze-drying to obtain dried polymeric networks (DPN) that after re-hydration give shape to spongy-like hydrogels[1, 2]. Different factors, such as, the freezing temperature, freezing rate and freezing time, have shown to affect the microstructural features of DPN and, consequently, the overall features of SLH[1,2,3]. Recurrent batch-to-batch variability suggested that other factors might affect spongy-like hydrogels preparation. Thus, in this work we aimed to further understand the influence of parameters, such as the diameter of the hydrogel (varying from 6 mm to 85.31 mm), the freezing temperature (-20ºC and -40ºC) and the freezing rate (0.1ºC/min and 1ºC/min), by using a freeze-dryer with a controlled and customized cycle of thermal treatment and drying steps. To attain this aim, GG DPN (0.75% and 1.5% (w/v)) were prepared and their microstructure was analyzed by micro-computed tomography and confirmed by scanning electron microscopy. As expected, there was a decrease of mean pore size with the increase of polymer amount, e.g. 138.20±29.52 µm (0.75% GG) vs 120.99±15.44 µm (1.5% GG) [0.1ºC/min, -20ºC, 6mm]. In respect to the influence of the diameter of the hydrogel, larger materials resulted in lower mean pore size, e.g. 138.20±29.52 µm (6 mm) vs 61.54±7.43 (22 mm) [0.1ºC/min, -20ºC, 0.75%]. Contrarily, the freezing temperature did not seem to affect the microstructure of the DPB, e.g. 120.99±15.44µm (-20ºC) vs 124.16±5.23µm (-40ºC) [0.1ºC/min, 1.5%, 6mm]. Disappointingly, conclusions regarding the effect of the freezing rate were not attained. Inconsistent data indicated that the equipment was not capable of reaching high freezing rates (1ºC/min). Nonetheless, this system could be of use to further tailor DPN microstructure according to specific applications for tissue engineering and to further standardize the processing of spongy-like hydrogels.

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NATURAL-BASED ELECTRIC-RESPONSIVE GELLAN GUM BIOINKS FOR MUSCLE REPAIR

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A bioengineered skeletal muscle tissue, which mimics the structural and functional characteristics of the native tissue, is needed for reconstructive surgery. Rapid progress in the cell-based tissue engineering principles have enabled *in vitro* creation of cellularized muscle-like constructs. However, the current fabrication methods are still limited to build a three-dimensional (3D) muscle construct with a highly viable, organized cellular structure with the potential for a future human trial. 3D bioprinting has appeared as an automated, precise and reproducible manner to develop more complex 3D functional living tissues. Thus, we are exploring the development of skeletal muscle-inspired bioinks composed of gellan gum hydrogels combined with natural-derived eumelanin nanoparticles (EuNPs) for better electrical and mechanical properties and loaded with C2C12 cell line (mouse myoblast cell line). This bioink was based on the use of gellan gum modified with divinyl sulfone, previously developed in our group, which allows covalent conjugation of thiol terminated cell adhesive peptides [1]. In brief, gellan gum was chemically conjugated with divinyl sulfone (GGDVS) by precipitation methods, which was confirmed by ^{1}H-NMR analysis. The peptides were added to GGDVS dissolved in sucrose solution. Then, EuNPs were purified by HCl to remove all residues from the ink sacs of cuttlefish and further mixed with peptide-conjugated GGDVS [2]. Using a BioX 3D bioprinter (CellInk), the different printing condition of this bioink, namely temperature, pressures, speeds, and pre flow rates have been optimized. Combined together, we will apply 3D bioprinting strategy to fabricate an implantable, bioengineered skeletal muscle tissue composed of C2C12 cell line for further development of 3D culture models.

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MICROGUIDED LASER ABLATION FOR THE BIOFABRICATION OF SKIN SUBSTITUTES WITH FOLLICULAR UNITS

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Commercially available skin substitutes represent a simplistic version of the native skin, far from replicating its complexity and main functions. The hair follicle (HF) strongly contributes to the most important physiological functions of the skin and is endowed with regenerative capacity. Thus, recreating HFs would represent a breakthrough in the development of clinically useful skin substitutes with improved integration and healing capacity for wound management, while allowing the construction of more reliable *in vitro* 3D models for drug screening. Herein, we used microscopy-guided laser ablation (MGLA) to elicit HF formation in a cultured dermal-epidermal skin construct. Hair forming units (HFUs) containing compartmentalized follicular dermal papilla cells surrounded by keratinocytes were included in a dermal equivalent (DE) and MGLA was used to create microchannels bridging the HFUs and the surface of the DE. Epidermal keratinocytes were then seeded on top and the standard procedure for the construction of bilayered skin construct was followed. Upon definition of the processing parameters (laser incidence area and power), MGLA manipulation of a DE layer of fibroblasts in collagen successfully guided the migration and integration of keratinocytes towards the HFU, in a process reminiscent of the initial steps required for HF development. Histological (H&E staining) and immunological (K15, K14, K10, ß-catenin, involucrin, vimentin and fibronectin) analysis of the constructs confirmed the formation of folliculoid structures that recapitulate the HF microphysiology and architecture within the skin construct. The biofabricated skin bearing follicular units opens new avenues in engineering skin substitutes with improved functionality while also representing a promising *in vitro* tool to study the mechanisms controlling HF development or for the screening of bioactive substances.

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MONOCYTE-DERIVED MACROPHAGE IMMUNE INTERACTIONS WITH GELLAN GUM-BASED HYDROGEL FORMULATIONS

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Gellan gum (GG) hydrogels have been widely used for tissue engineering and regenerative medicine approaches with minimal biocompatibility issues [1]. Biofunctionalization of GG with cell adhesive sites has shown improved in vitro cell response [2], but how it affects the immune response remains to be elucidated. Therefore, in this work, we intended to evaluate the impact of the absence/presence of cell adhesive sites on human monocyte-derived macrophages (MDM) response. MDM, differentiated from primary monocytes sorted from peripheral blood mononuclear cells, were encapsulated in GG hydrogels with or without the cell adhesive site RGD [2]. Cells showed a round-shape morphology in non-adhesive hydrogels and a spread adhesive-like morphology in RGD-containing hydrogels, while cell viability up to 12 days of encapsulation was not affected. A strong release of both proinflammatory (IL-1ß, IL-6, IL-12p40, TNF-α) and antiinflammatory (IL-1RA, IL-10, CCL22) associated cytokines was observed 1 day after MDM encapsulation, independently of the hydrogel formulation. This release on day 3 was strongly reduced for most cytokines to undetectable levels for TNF-α. Conversely, CCL17, which was undetectable on day 1, was detected on day 3 even if at very low levels, and CCL22 release slightly increased. A dependence on the hydrogel formulation was observed, as higher amounts of proinflammatory cytokines (IL-1ß and IL-12p40) were detected from MDM within soft hydrogels lacking RGD, whereas a higher release of antiinflammatory cytokines (IL-1RA, IL-10, CCL17, and CCL22) was detected from MDM within RGD-containing hydrogels. Overall, this work reveals that MDM presented an immediate but transient response to GG hydrogels, which is affected by its mechanical properties and the presence of a cell-adhesive site.

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ECO-FRIENDLY AND BIOCOMPATIBLE ENZYMATICALLY CROSS-LINKED SILK HYDROGELS FOR 3D IN VITRO MODELING AND CANCER RESEARCH

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The incidence of cancer in the world has been increasing, which has a major impact on the global healthcare system. Tumors like glioblastoma or osteosarcoma, not only present high metastases rates as the current treatments (e.g., chemotherapy) do not completely satisfy clinic outcomes. Tissue engineering strategies emerged as sustainable solutions for the diagnosis and treatment of cancer. This work aims to develop novel and eco-friendly hydrogel-based *in vitro* models reflective of tumors heterogeneity and microenvironment. The concept is based on developing more efficient silk fibroin (SF) hydrogels immobilizing human carbonic anhydrase's (CAs) (HRP-SF/CA), following the horseradish peroxidase (HRP)-mediated cross-linking that reacts with the tyrosine groups (~5 mol%) on SF and CA (~4 mol%)[1]. CAs catalyze the reverse hydration of CO_2 to produce HCO_3^- and H^+ regulating the pH and metabolic rates of tissues. Moreover, it was found that CA is part of the natural spinning process of fibroin from spiders and silkworms inducing ß-sheets' formation [2]. Thus, it is expected that the immobilization of different isoforms of CAs induce a strong modular character to HRP-SF hydrogels according to tissue requirements and tumor disease modeling. In fact, the catalytic activity of CA-based materials has been proposed for the sequestration of CO_2 under mild conditions, which would be highly valuable for controlling carbon emissions to the environment [3]. Herein, the cytosolic CA type II (CAII) was proposed for its role in metabolic regulation of tissues like bone and upregulation in tumors like glioblastoma [4]. The HRP-SF/CAhydrogels showed higher ß-sheet content and crystallinity as compared to control HRP-SF hydrogels, according to the CAII concentration (CA-II: 50–450 µg/ml) and oxidizing agent (H_2O_2, CaO_2). Moreover, a positive influence of CA immobilization was observed on U251 glioblastoma cancer cells encapsulated within the matrices up to 7 days of culture. High CA-immobilization efficiency was observed in all tested conditions, and controlled drug release profile from Doxorubicin-loaded HRP-SF/CA hydrogels. Although further studies are necessary to maturate the potential of the HRP-SF/CA matrices to recreate the hypoxic microenvironment of solid tumors *in vitro*, the preliminary data suggest desirable physicochemical properties of the hydrogels to be further used as bioinks in the design of complex 3D *in vitro* cancer models.

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A MICROREACTOR FOR THE PREPARATION, MAINTENANCE AND CONDITIONING OF MULTILAYER TISSUES OR MULTI-TISSUE STRUCTURES

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Dynamic culturing systems can contribute to overcoming challenges of *in-vitro* fabrication and maintenance of complex 3D tissues, however, the unique physiological conditions to which each tissue is subjected has been hampering noteworthy developments for many engineered tissues. Here we report the development (design, manufacturing and testing) of a micro bioreactor for the preparation, maintenance and/or conditioning of human multilayer tissues or multi-tissue structures, providing evidence for vascularized skin tissue-engineered analogues and ex-vivo human skin. The bioreactor comprises a sandwich modular structure of hard undeformable layers of medical grade polycarbonate intercalated with soft deformable layers of silicone. When compressed the soft layers expand laterally against the sample sealing the layers between fluid streams avoiding their intermixing. Furthermore, the bioreactor is modular so that by changing the thickness of the soft layers it can easily be adapted to accommodate samples with varied thicknesses. The bioreactor is capable of holding skin tissue samples of 8mm in diameter and of providing, without mixing, different culture media corresponding to the three layers of the tissue, the outmost epidermis, the underneath dermis and the innermost adipose tissue. This feature allows nourishing each cell type/tissue layer with the specific cell culture medium, increasing the maintenance time of the native structure in the ex-vivo skin. Furthermore, the bioreactor permits establishing an air-liquid interface for the epidermis turnover in the skin explant, while still maintaining the separation of the culture media underneath. This dynamic culture system contributes to diminishing the time of preparation of complex tissues or multi-tissues and prolonging the viability and use of in vitro and ex-vivo tissues being, therefore, a valuable tool for drug discovery, personalized medicine and cancer development studies.

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EVALUATION OF OSTEOGENIC POTENTIAL OF SPONGE-DERIVED BIOSILICA FOR BONE TISSUE ENGINEERING APPLICATIONS

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The use of biosilica derived from marine sponges became very attractive in the bone tissue engineering (TE) perspective [1]. It has been shown that biosilica can stimulate the osteogenic differentiation of stem cells and increase the mineralization of osteoblast-like cells [2]. In our study we evaluated the ability of biosilicas derived from five North Atlantic deep-sea sponges, *i.e. Stelletta normani* (SN), *Phakellia ventilabrum* (PV), *Axinella infundibuliformis* (AI), *Geodia atlantica* (GA) and *Geodia barretti* (GB) to promote osteogenic differentiation. The biosilicas were obtained by sponge calcination at 800°C for 6 hours. Bone marrow Mesenchymal Stem Cells (hBMSCs) were cultured in the presence of leachables from the different biosilicas (generated at a concentration of 10 mg/mL) in both basal and osteogenic differentiation medium (basal medium supplemented with 50 mg/mL of ascorbic acid, 10^{-2} M of beta-glycerophosphate and 10^{-7} M dexamethasone) for 7, 14 and 21 days. DNA quantification analysis confirmed the proliferation of hBMSCs in all culture conditions and time points. The analysis of the ALP protein expression showed that the ALP values for GA-derived biosilica increased with time under basal conditions. Under osteogenic conditions the protein activity for GA-derived biosilica is significantly higher at 14 day of cell culture. From RT-PCR data it was observed the upregulation of three different genes, Runx2, osteopontin (OP) and ALP under osteogenic conditions. Runx2 is considered an early marker of osteogenic differentiation, its upregulation is observed at day 21 in cells cultured under osteogenic conditions. The bone non-specific gene ALP, which is considered an early-stage indicator of osteogenic differentiation, is observed for the cells when in the presence of all tested biosilicas. Finally, OP a late marker of osteogenic differentiation shows upregulated expression mainly at day 14 and 21. PCR data showed, that the GA-, GB- and AI-derived biosilicas present promising results for the promotion of osteogenic differentiation. The immunofluorescence of OP protein expression by hBMSCs was observed at early time point (7 days) for all samples. In contrast to basal conditions, OP expression is higher for all samples under osteogenic conditions. The obtained results showed the potential of biosilicas from marine sponges for the development of biomaterials for bone TE applications.

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DEVELOPMENT OF LASER ABLATION-COMPLIANT HYDROGELS FOR TISSUE ENGINEERING

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One of the main areas of focus in the Tissue Engineering field is the development of three-dimensional (3D) matrices to support cells and their activity, mimicking the native extracellular matrix (ECM). Hydrogels, especially the ones made from ECM proteins, have shown great promise to fit this role due to their similarity with native ECM in terms of chemistry, water content and mechanical properties. These and other properties can be tuned by varying the hydrogels' components or their concentrations. This tunability is very important in the development of efficient methods for their structural modification (e.g. micropatterning) to improve and control cell behaviour. Laser ablation is an emerging tool for creating user-defined architectures with high resolution control in hydrogel-based 3D matrices. This technique is very sensitive to the optical properties of hydrogels. In fact, turbidity, a common outcome in hydrogels based on ECM proteins such as collagen, impedes efficient ablation. The objective of this work was to investigate the compliance of several gelatin- and collagen-based hydrogels with the laser ablation process. For that, the optical and rheological properties of several formulations of gelatin, collagen and collagen-gelatin hydrogels were assessed. The same hydrogels were then subjected to different laser ablation protocols. Data showed that only highly transparent hydrogels could be successfully ablated. A certain degree of stiffness was needed for the hydrogels to maintain the ablated 3D structures. Crosslinked gelatin hydrogels were shown to be the easiest to ablate, demanding less laser intensity to create hollow structures inside the 3D matrix. This work provides a starting point to better define hydrogel formulations for efficient laser ablation.

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SURFACE ENGINEERED POLYURETHANE URETERAL STENTS: A NATURE-BASED APPROACH TO ENHANCE ANTIBACTERIAL PROPERTIES

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Bacterial colonization of ureteral stents leads to a severe and challenging clinical complication that has drawn widespread concern in the last decades. As so, medical and patients' communities demand for effective answers to this challenge. In this context, we present a strategy based on the functionalization of ureteral stents with chitosan-fatty acids derivatives (CS-FA) to prevent bacterial stent colonization. Three different fatty acids, saturated or unsaturated fatty acids, namely stearic acid, oleic acid, or linoleic acid, were grafted onto chitosan polymeric chain to produce CS-FA derivatives that were fully characterized at a physicochemical level. The CS-FA derivatives-based coatings were further developed on the surface of polyurethane (PU) ureteral stents via carbodiimide-mediated coupling reaction. The presence and homogeneity of the CS-FA derivatives-based coatings onto the surface of PU stents were confirmed using X-ray photoelectron spectroscopy (XPS) and crystal violet staining. The antibacterial potential of CS-FA coated PU stents were evaluated against several microorganisms, including *Escherichia coli*, *Proteus mirabilis*, and *Methicillin-resistant Staphylococcus aureus*. Alamar blue method was employed to quantify the bacterial attachment onto CS-coated PU stents and CS-FA-coated PU stents, being the data normalized by the control (i.e., PU stent). To further confirm such antibacterial effects, Scanning Electron Microscopy (SEM) was used to evaluate the morphology of the bacteria adhered to the surface of untreated PU stents and CS and CS-FA derivatives coated stents. Bacterial colonization assays illustrated that untreated PU stents did not prevent bacterial colonization, while in coated stents, a reduction on the number of bacteria attached to the surface is observed. Cytotoxicity studies were also performed for all CS-FA coated PU stents confirming the existence of a negligible cytotoxicity.

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DEVELOPMENT OF A BLOOD BRAIN BARRIER MODEL USING CELL-SHEET ENGINEERING

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The incidence of brain pathologies including cancer is continuously growing in the modern society. Such pathologies are still challenging for treatment as an efficient therapeutic delivery is impeded by a selective physiological barrier - the blood-brain barrier (BBB). BBB is a dynamic interface between the central nervous system (CNS) and the circulating blood restricting the transport of toxic or harmful molecules from the blood to the brain, and allowing transfer of nutrients to the brain and removal of metabolites. The physiological and pathological importance of the BBB has led to the development of different *in vitro* models to study the dynamics of this barrier and to validate the efficacy of new therapeutic solutions. The majority of these models are based on culture of brain endothelial cells or co-culture of these cells with other cells from the neurovascular unit, such as astrocytes, pericytes and/or neurons. Although useful, these models are limited in recapitulating the organization and signaling in the native BBB. Herein, we propose a model based on cell sheet engineering (CSE) and co-culture of brain microvascular endothelial cells (hBMECs) with astrocytes and pericytes. CSE, traditionally applied for tissue and organ regeneration, allows the creation of 3D complex constructs by layering cell sheets (of the same or different cell types) in which cell-cell connections and extracellular matrix (ECM) are preserved[1]. We first obtained cell sheets from the different cell types separately (hBMECs, pericytes and astrocytes) and characterized the expression of specific markers by immunocytochemistry. Then, a co-culture of hBMECs over pericytes was tested to evaluate the formation of a handleable cell sheet and the maintenance of specific markers (Claudin5, SMA). Finally, we build the cell sheets by consecutive seeding of astrocytes, then pericytes and finally, hBMECs on top of the construct. We were able to obtain confluent cell sheets that were preserved upon detachment. The live/dead staining of the sheets showed high cell viability. Immunocytochemistry revealed the expression of specific markers (Claudin5, SMA, GFAP) for the different cell types and histological analysis of the stratified astrocytes/pericytes/hBMEC cell sheets revealed the existence of different layers of tightly packed cells in a collagen matrix.

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MARINE-BASED NANOPARTICLES WITH ERBB-2 ANTIBODY IMMOBILIZED TARGET BREAST CANCER CELLS BOTH IN VITRO AND IN VIVO

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An effective anti-cancer therapy aims to eliminate tumor cells without toxic effects on the surrounding healthy tissues. Since current chemotherapeutic drugs may present severe side effects, great efforts have been made in the development of alternative and well-tolerated therapies. The targeting of specific receptors present in cancer cells is an interesting approach to develop successful cancer treatments. Since ErbB-2 (human epidermal growth factor receptor 2) is overexpressed in around 30% of breast cancers, the respective antibody was immobilized at the surface of marine-polymeric nanoparticles (NPs). Fucoidan/chitosan NPs incorporating gemcitabine (NPs+Gem) were produced by polyelectrolyte complexation as previously optimized by our group, and their surface functionalized with ErbB-2 antibody (NPs+Gem+Ab) [1]. The maximum antibody immobilization was optimized as 10 μg mL^{-1}. NPs+Gem+Ab present size of 159±23 nm, a polydispersive index of 0.178±0.027, and a zeta-potential of 21.30±2.68 mV, indicating that nanoparticles are monodisperse and stable. The targeting ability of NPs with immobilized ErbB-2 antibody was validated by the increased cellular uptake of SKBR3 cells (ErbB-2 positive breast cancer cells) as compared to MDA-MB-231 (ErbB-2 negative breast cancer cells). To further validate the efficacy of the targeting, a co-culture system was established with human endothelial (EA.hy.926) and breast cancer cells (SKBR3). Similar cytotoxicity was observed for Gem, NPs+Gem, and NPs+Gem+Ab concerning the effects on endothelial cells after 24h (between 24-30%). Additionally, NPs+Gem+Ab presented toxicity to breast cancer cells around 80%, whereas free Gem and NPs+Gem only presented toxicity around 12% and 23%, respectively, confirming the efficacy of the targeting system. In an *in vivo* study, where SKBR3 cells were injected into the mammary fat pad of immunocompromised mice, NPs+Gem+Ab induced tumor growth impairment. Furthermore, tumorigenic mice treated with NPs+Gem+Ab presented less metastatic lungs. The targeting efficacy of the NPs+Gem+Ab system was validated both *in vitro* and *in vivo*, being the developed system a possible approach for ErbB-2 positive breast cancer treatment.

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P25

COPOLYMERS WITH HYALURONAN BRANCHES AS EFFICIENT ANTAGONIST OF CD44 SIGNALLING IN BREAST CANCER CELLS

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CD44 is the main hyaluronan (HA) receptor involved in the communication of cells with their microenvironment. CD44/HA interactions and following signaling cascade(s) depend on HA size. In cancer, HA with high molecular weight interacts, organizes, and clusters several extracellular and membrane proteins, including CD44, to form a dense and functional pericellular coat.[1] These interactions activate anti-apoptotic, pro-invasion, and migration signaling pathways, promote drug resistance, facilitate intravasation, protect against anoikis during circulation, and contribute to ectopic tissue colonization.[2] Inhibition of such CD44/HA interactions and CD44 clustering has been thus explored as a therapeutic target.[3] In this work, we synthesized poly-(hydroxyethyl)methacrylate graft HA (HEMA-g-HA) by oxime reaction at the reducing end of HA (4.8 kDa). We studied the interactions of these copolymers with CD44 by Surface Plasmon Resonance (SPR) and compared them against HA of low (4.8 kDa) and high (1.35 MDa) molecular weight. All copolymers bind to CD44 with the high association and dissociation constants: we observed a much higher affinity of CD44 to HEMA-g-HA than to HA (4.8 kDa) and similar to the measured for HA (1.35 MDa). On the other hand, the degradation of HEMA-g-HA by hyaluronidase is slower than free HA as shown by isothermal titration calorimetry (ITC). These results indicated that HEMA-g-HA could be a good antagonist of CD44/HA signaling in cancers. To check this hypothesis, we studied the binding of HEMA-g-HA-Rho to GFP-CD44 in transfected Sk-Br-3 cells by Förster resonance energy transfer (FRET) microscopy. FRET showed no significant difference between the copolymer, HA of 4.8 kDa, and 1.35 MDa HA, suggesting the same binding sites. Competitive binding assays demonstrated similar activity for HEMA-g-HA and HA of 4.8 kDa, but Western blot and immunocytochemistry analysis evidenced that the copolymer regulates CD44 expression and location, promoting receptor declustering and translocation to the cytoplasm - alternations that ultimately lead to cell death.

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3D MODELS RECAPITULATING A BONE MICROENVIRONMENT CAPABLE OF PROMOTING THE DI FFERENTIATION OF OSTEOBLASTS TOWARDS OSTEOCYTES

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Researchers have been making significant efforts towards engineering new 3D *in vitro* bone models. It is well known that osteocytes play a vital role in maintaining bone homeostasis, since they are around 90% of the bone constitution (1,2). The number of current 3D *in vitro* models capable of promoting the differentiation of osteoblasts towards osteocytes, and that faithfully recapitulate a bone microenvironment, is limited. Besides, in the existing models, human cells do not prevail over animal cell lines. In this study, we propose a new and more realistic 3D *in vitro* bone model for a better understanding of bone physiology and disease. An effective differentiation into osteocytes is the targeting strategy of our 3D model, which is composed of Gellan Gum-Hydroxyapatite (GG-HAp) spongy-like hydrogels and human osteoblasts (hOBs) under osteogenic conditions and long-term culture. To this end, the metabolic activity, proliferation, viability, morphology, and spatial rearrangement of hOBs entrapped within GG-HAp spongy-like hydrogels were assessed by resazurin assay, DNA quantification, live/dead, phalloidin/DAPI, and H&E stainings, respectively. In addition, the hOBs ability to support osteocyte differentiation through the detection of mature osteoblastic and osteocytic genes was evaluated by RT-PCR. We observed that hOBs expressed the target genes (ALP, Runx2, COL I, OC, OPN, OSX and hPDPN) and migrated, attached, and spread throughout the GG-HAp matrix. The newly formed tissue was also mineralized. Overall, our 3D *in vitro* bone model holds a great promise for a better understanding and diagnostic of various bone diseases.

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DEEP LEARNING IN BIOENGINEERING: BIOFABRICATION AND 3D PRINTING TECHNOLOGIES

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The average life expectancy is increasing leading to increased demand for organ replacement and transplantation that results in the constraint of organ availability [1]. Bioengineering have been tackling this unmet clinical need by attempting to develop biofunctional tissues in the laboratory. Current advances in this field have been made focusing in revolutionized biofabrication strategies, especially 3D bioprinting, leading to great advances and to the engineer of approaches capable to mimic native tissues with more consistency and cost-effectiveness. Nevertheless, these promising technologies are still far from the clinical setting. Deep learning, as a subset of artificial intelligence, can be used to bring bioengineering strategies closer to the patients. Deep learning technology can autonomously use massive datasets to produce valuable outputs in an intelligent and effective manner. Deep learning can be a powerful tool in bioengineering approaches and the synergy of this technology and biofabrication will help to close the gap between 3D bioprinting to the clinics[MOU1] . This work provides an overview of the use of deep learning in bioengineering and biofabrication with special focus on 3D printing.

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INSIGHTS ON THE CORROSION AND ENCRUSTATION OF BIODEGRADABLE MG ALLOYS IN URINARY TRACT ENVIRONMENT

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Biodegradable metals have unique features that make them attractive for biomedical applications, including an increased radial force and slower degradation rate, when compared with polymeric biodegradable materials. Indeed, these are excellent properties for ureteral stents since the increased radial force will be suitable for clinical cases with high external compression (e.g., tumour) and the slower corrosion allows their use on biodegradable ureteral stents for prolonged time treatments. Notwithstanding, the exploration of biodegradable metals for urological application is scarce, even though these are considered promising materials. In this sense, this work focuses on the exploration of the biodegradable metal's behaviour under *in vitro* urinary tract environment. Five biodegradable Mg alloys were selected: AZ31, Mg-1Zn, Mg-1Y, Pure Mg and Mg-4Ag, and studied under static and dynamic conditions with artificial urine solution (AUS). A corrosion layer was formed at the surface of all the samples. The chemical analysis of this layer was accessed by SEM/EDS, XPS and Raman and the results indicated that the composition is similar for all the samples and the prevailing elements were O, C, P, Mg and N, which were attributed to struvite ($NH_4MgPO_4{\cdot}6H_2O$), hydroxyapatite ($Ca_{10}(PO4)_6(OH)_2$) and magnesium hydroxide ($Mg(OH)_2$), as revealed by Raman. For the metal´s corrosion analysis, the samples were first submitted to a cleaning step in order to remove the corrosion layer formed during AUS immersion. The corrosion rate of the samples was calculated based on the weight loss along the assays. It was found that Mg-4Ag and Pure Mg had the higher corrosion rate and the metals corrode differently, i.e. in AZ31 the corrosion decreases along the time while in pure Mg increases, for instance. Additionally, it was found that dynamic conditions accelerate the corrosion, even though this trend was not significative for all the metals. The profilometry and SEM data showed that the samples have a different corrosion pattern, some presented huge corrosion holes, while others presented various agglomerates of several small pitting holes. It should be noted that most of the samples, with the exception of Mg-1Y, presented a non-homogeneous surface, which is not desirable for ureteral stents. Therefore, the non-homogeneity together with the observed tendency for encrustation are problems that are being accessed through optimizations on the metal alloys' production.

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BIOPHYSICAL PROPERTIES REGULATE COATINGS' PERFORMANCE IN NEURONAL CULTURE ACCORDING TO A PERFORMANCE FACTOR-BASED MATHEMATICAL MODEL

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Coating of adhesion proteins is an essential step for culture of sensitive adherent cells, such as neurons. In this work, we demonstrate the major contribution of physical properties of coatings in neuronal culture using a highly reproductible methodology. To do so, SH-SY5Y cells neuronal differentiation on biomaterial's coatings was used as an evaluation of neuronal culture, with extension and number of neurites formed as performance parameters. The surface physical properties measured were z-potential (ZP) and water contact angles (WCA), known to be crucial in protein folding and adsorption. These properties were measured in a library of biomaterials' coatings and cells were seeded onto these. A non-linear mathematical model was developed to correlate the biphysical and biological measurements, searching for two differently performing sets of biomaterials. Two critical values of WCA and ZP were found, 82° and -26.43 mV, respectively, which define a border between a high and a low biological performance domain for a determined WCA value. These two clusters were identified achieving correlation factors R^2 of 0.92 and 0.90 for total neurite's length and number of neurites, respectively, showing a robust prediction. Thus, hinting at biophysical protein adsorption mediated neuronal adhesion, this work establishes the basis for biomaterials screening and selection for both, 2D and 3D cell culture. This model could be used to pre-determine the performance of coated surfaces, and in particular, may assist the optimization of layer-by-layer methods for neural culture and differentiation.

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DEVELOPMENT OF PLASMONIC POLYMERIC BASED MEMBRANES FOR LOCALIZED SURFACE PLASMON RESONANCE BIOSENSING

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Localized Surface plasmon resonance (LSPR) phenomenon occurs when incident light interacts with metallic nanoparticles that have smaller sizes than the wavelength of the incident light. LSPR technology has gain the attention of several biosensing applications, since it can provide a tool for highly sensitive, flexible, rapid, label-free, and real time monitoring of molecular interactions. Nevertheless, sensing devices should also be biocompatible, flexible, miniaturized, in order to realize the bio-related detection or on-site health monitoring systems. In LSPR-based biosensors, the refractive index (RI) changes, induced by biomolecular interactions, affects the optical signal and these changes can be measured by tracking the resonant feature in the spectrum of scattered or transmitted light. [12] In this work, we developed plasmonic membranes composed of gold nanostructures embedded in a chitosan matrix. To optimize the biosensor sensitivity, several membranes with different concentration of chitosan and with gold nanorods and gold nanoparticles, were produced, to understand the influence of the plasmonic nanostructures and the polymer concentration in the biosensor sensitivity. A high resolution LSPR spectroscopy system was used to detect changes in RI of liquids, at room temperature. Membranes with lower polymer concentration presented better results achieving a refractive index sensitivity (RIS) to bulk RI changes up to 77 ± 4 nm/RIU. The final objective of this research is to use these biocompatible and flexible LSPR biosensors to perform a rapid and sensitive detection of relevant biomarkers of high incidence diseases, such as cancer, sepsis and cardiovascular diseases biomarkers.

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STATE-OF-THE-ART AND FUTURE OF BIOINKS IN 3D BIOPRINTING

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Three-dimensional (3D) bioprinting is a term regrouping all additive manufacturing techniques used in tissue engineering. These techniques aim to produce living 3D structures from mixtures of cells and materials with natural and/or synthetic origins known as bioinks. This field comes at the convergence of additive manufacturing, tissue engineering and regenerative medicine, aiming to overcome the limitations faced by traditional biofabrication techniques. From its premises in 1988, when Klebe modified a standard Hewlett-Packard printer to deposit cells by a process then known as cytoscribing [1], 3D bioprinting has come a long way to now produce structures that can be implanted *in vivo* as part of therapeutic procedures, used for *in vitro* cell culture, as models for pharmaceutical research, etc [2]. 3D bioprinting especially represents a promising solution to global tissue and organ shortage. Beside the development of bioprinting techniques and hardware, bioinks have also greatly evolved from the simple cell media capable of supporting the bioprinting requirements they once were. Bioinks are now being engineered to mimic specific native tissues characteristics in terms of mechanical properties, biofunctionality and microstructure, while still acting as an encapsulation media which can safely deliver cells and other therapeutics *in vivo*. Recently, bioinks have also been designed to integrate further functionalities, such as controlled degradation, shape morphing, *in vivo* printability, and more. The current state-of-the-art and research trends in bioink engineering are overviewed herein.

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EXTRACELLULAR MATRIX - DERIVED HYDROGELS FOR TISSUE ENGINEERING

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Tissue engineering (TE) is an interdisciplinary field that creates biological alternatives to restore or enhance the function of damaged tissues or organs. One critical aspect in creating such alternatives for perfused tissues is their vascularization since cell necrosis is a common outcome if the diffusion of oxygen and nutrients is not assured. Incorporating angiogenic chemical cues in these models may be a potential solution for this problematic. In this line, adipose tissue-derived stromal vascular fraction (SVF) is a heterogeneous cell fraction that was shown to undergo spontaneous vasculogenesis in vitro due to enabling growth factor secretion and extracellular matrix (ECM) composition. The ECM is an organized network composed of several macromolecules and comprises the non-cellular component of living tissues. This network functions as a 3D matrix with a unique chemical composition that supports cellular activities and responses required for tissue homeostasis. When developing TE constructs, it is crucial to mimic as much as possible native ECM in order to better approximate the cellular environment to the native tissue dynamics. Hydrogels are well-established tools that can be used as ECM surrogates to provide mechanical support and biological cues and direct cell behavior. Therefore, the aim of this work was to achieve an angiogenic/vasculogenic hydrogel derived from SVF-derived ECM. We were able to isolate ECM from cell sheets made of SVF-derived adipose stem cells (ASCs) and develop an ECM-like hydrogel. ASCs cell sheets were decellularized by a combination of freeze-thaw cycles and a nuclease treatment. The samples were freeze-dried and digested with an acidic pepsin solution, and a hydrogel was formed after pH neutralization and temperature increase. Through circular dichroism (CD), we were able to detect the triple helix structure of proteins, confirming protein structural stability after the extraction protocol. Additional studies are ongoing to better characterize the obtained hydrogel and assess its angiogenic/vasculogenic potential. If successful, this strategy of obtaining angiogenic/vasculogenic ECM-like hydrogels has the potential to be used in a broad range of different applications in TE and biomedical research.

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NEXT GENERATION BIOINK BASED ON MINERALIZED SHARK COLLAGEN WITH INTRINSIC OSTEOGENIC PROPERTIES: A STEP TOWARDS THE FUTURE OF BONE REGENERATION

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3D printing with incorporated living cells – bioprinting – has gained special attention on tissue engineering approaches, aiming to closer recapitulate the target tissue microenvironment. However, it raises additional complexity related with the need to use cell-friendly printing conditions that still comply with material printing fidelity. Inspired on the composite nano structural organization of mineralized tissues, this work reports the efficiency of the chemical approach followed to *in situ* mineralize blue shark skin collagen, at a nano scale level, to ultimately produce stable inks. The impact of initial cellular density was evaluated by testing three different concentrations (2.5, 5 and 7.5 $x10^6$ cell.ml^{-1}) of human adipose stem cells (hASC), where biological results demonstrated improved cell viability with the higher density of encapsulated cells. Immunodetection of RUNX2 and Osteopontin 21 days after cell culture confirmed the potential of the ink for osteogenic differentiation without any exogenous stimulation, which may be due to successful cell-to-ink interaction and the Ca^{2+} ions released from the co-precipitated hydroxyapatite. A combination of mineralized shark collagen, alginate and hASC is thus proposed as bioink with potential properties for the regeneration of bone tissue.

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DEVELOPMENT OF A BIOACTIVE FIBROUS SCAFFOLD WITH OSTEOINDUCTIVE PROPERTIES

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The surface chemistry of tissue engineering scaffolds provides better cell adhesion and influences cell fate. It can introduce chemical cues for cell triggering signalling pathways through protein adsorption to control desired tissue growth. Hence, enhancing the cell–scaffold interactions by surface functionalization is a valid strategy to achieve better tissue integration and improved tissue healing. Therefore, this study aims to define a method for the development of an osteoconductive and osteoinductive scaffold. To do so, electrospun fibrous meshes (eFMs) produced with polycaprolactone will be functionalized with a silane linker able to immobilize an osteogenic factor. 3-aminopropyl triethoxysilane (APTES) is a silane linker that will act as a spacer, allowing more steric freedom of the immobilized biomolecule. Moreover, *in vitro* and *in vivo* studies demonstrated that Si has a positive impact on bone homeostasis by taking a role in early calcification processes. Bone morphogenetic protein-2 (BMP2) has high osteoinductive properties and plays a crucial role in bone formation and repair. However, its high cost and short half-life, as well as the unspecific side effects in other body regions, increases the risks associated with high dose BMP2 clinical treatments. The morphology and chemical composition of eFMs activated by UV-Ozone and functionalized with APTES by vapour deposition were evaluated by SEM and XPS, respectively. The osteoinductive capability of APTES-functionalized eFMs was confirmed by immersion tests in SFB. The osteogenic potential of the functionalized eFM was assessed by culturing human bone marrow-derived mesenchymal stem cells (hBM-MSCs) in basal and osteogenic media. The results show that the APTES induced an increased cell adhesion and proliferation in comparison to bare eFMs. Further studies will be conducted to assess the binding of BMP2 to APTES- functionalized eFMs and the osteoinductive property of this fibrous scaffold. An osteoconductive and osteoinductive system will be developed envisioning an effective bone regeneration.

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DEVELOPMENT OF DIFFERENT FORMULATIONS OF MANGANESE DIOXIDE-BASED NANOREACTORS FOR APPLICATION IN MRI IMAGING AND OXIDATIVE STRESS REDUCTION

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The application of nanoparticles in magnetic resonance imaging (MRI) and therapeutic approaches targeting inflammation has been greatly increasing, due to their advantageous properties such as nanoscale dimension and tuneability [1]. In this context, manganese (Mn^{2+})-based nanoparticles have been greatly investigated, due to the critical role of these ions in brain health, and to their valuable use as a contrast agent, improving signal intensity and specificity in MRI (manganese-enhanced MRI, MEMRI) [2]. Additionally, Mn^{2+} can act as scavengers of reactive oxygen species (ROS), commonly present in the inflammatory processes of neurodegenerative diseases [3]. The aim of the present study was to develop nanoreactors, which can be used not only as ROS scavengers, to reduce apoptosis and inflammation in tissues, but also as contrast-agent in MEMRI. Several blends of methacrylated gellan gum (GG-MA) and hyaluronic acid (HA) were embedded with different types of manganese dioxide (MnO_2) nanoparticles and further physico-chemically characterized. Dynamic light scattering, scanning electron microscopy, water uptake and degradation studies were performed. *In vitro* cytotoxicity of the different formulations was also evaluated using an immortalized rat fibroblast cell line L929, up to 72 h of culturing. Synthesized nanoparticles were obtained with an average size of 70 nm and round-shaped morphology. The stability of the different formulations of hydrogels was not affected by nanoparticles' concentration or HA ratio. The presence of synthesized MnO_2 (MnO_2_S) nanoparticles reduced hydrogels' cytocompatibility, whereas the commercially available type 1 (MnO_2_C1) nanoparticles were less toxic to cells. Additionally, cell proliferation and viability were enhanced when a lower content of HA was present. Higher concentrations (75 and 100 ng/mL) of MnO_2_S and MnO_2_C1 nanoparticles did not negatively affected cell viability, whereas the opposite effect was observed for the commercial type 2 (MnO_2_C2) nanoparticles. Further studies are required to evaluate the potential application of the most promising nanoreactors' formulations for combined application in MEMRI and as ROS scavengers.

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FABRICATION OF EXTRACELLULAR MATRIX-ENRICHED CELL SHEETS DERIVED FROM THE STROMAL VASCULAR FRACTION OF HUMAN ADIPOSE TISSUE

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The high demand for donated organs vastly outnumbers the supply, leading to the suffering of millions of patients in need. Tissue and organ engineering can be a possible solution to overcome this problem. However, prevascularization strategies to ensure irrigation of complex engineered tissues after transplantation continues to be challenging. The stromal vascular fraction (SVF) of human adipose tissue possesses a strongly angiogenic character due to its cellular composition. Previously, we have developed cell sheets of human SVF capable of undergoing spontaneous vasculogenesis *in vitro* in the absence of added growth factors, combining the cellular variety of SVF with the extracellular matrix (ECM) richness of cell sheets. In the present work, we aimed at defining optimal culture conditions of SVF cell sheets for downstream extraction and characterization of angiogenic ECM. SVF cells were cultured up to 32 days in the presence of different concentrations of ascorbic acid (AA) to boost ECM production. In addition, since cell sheet detachment is a common outcome of prolonged *in vitro* culture, a Rho-associated kinase inhibitor (ROCKi), which modulates the cytoskeleton and stress fiber formation, was also added to the culture. As expected, the presence of AA resulted in higher collagen levels after 25 days of culture, which was also true for fibronectin. Furthermore, the addition of ROCKi did not inhibit protein levels and, in fact, increased the detected levels of laminin. Additional proteomic characterization is under way. This work is the first step towards defining a protocol for producing ECM-enriched SVF cell sheets for downstream ECM extraction.

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A PERSONALIZED BIOACTIVE FIBROUS MEMBRANE CAPABLE TO PROMOTE THE FUNCTIONAL RECOVERY OF INJURED CAVERNOUS NERVE

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Prostate cancer is one the most common cancer type worldwide, affecting more than 16% of men. Radical prostatectomy (RP) is the main curative treatment of localized cancer. However, 60-70% of the patients will suffer from postoperative erectile dysfunction (ED) due to the unintentional cavernous nerve (CN) damage. In this study, a personalized bioactive fibrous membrane was proposed for the permanent treatment of prostate cancer patients suffering from ED after being subject to RP. The bioactivity of the fibrous membrane is conferred by the selectively bound nerve growth factor (NGF) present in the rat urine. The safety and efficacy profile of this implantable device to regenerate injured CN was investigated *in vivo*. In the rat model, the bioactive fibrous membrane improves recovery of the cavernous nerve and restores erectile function within one month after bilateral CN crush. Such functional recovery was confirmed by: (*i*) a high score of penis glans exposure and erection; (*ii*) a 60% increase in the intracavernosal pressure; (*iii*) a low score of cavernous nerve atrophy and irregularity; (*iv*) a low score of fibroelastic tissue density and vascular lesion in corpus cavernosum tissue and (*v*) a low score of dorsal nerves demyelination and degeneration. Our data suggests that the developed bioactive fibrous membrane constitutes a neuroregenerative approach able to promote erectile functional recover. Therefore, this personalized regenerative strategy could overcome the recognized drawbacks of currently available treatments of CN injuries. This implantable device represents a new hope for prostate cancer patients suffering from ED after being subject to radical prostatectomy.

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BIOMIMETIC SURFACE TOPOGRAPHY AS A POTENTIAL MODULATOR OF MACROPHAGES INFLAMMATORY RESPONSE TO IMPLANTED BIOMATERIAL SCAFFOLDS

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The main role of biomaterial scaffolds in tissue engineering strategies is to promote functional tissue growth. Several processes including the injury incurred during the implantation process and the associated host inflammatory response can negatively impact the local environment compromising the implantation success [1,2]. Immune cell responses to implanted biomaterial scaffolds mediate all subsequent host-biomaterial interactions[1,3], being essential for the process of biological integration[2-5].The immune system plays important roles in biological processes required for the integration of biomaterials such as wound healing, host tissue integration, inflammation, and foreign body reactions. Thus, it is essential to design immune-modulatory surfaces that can influence host responses towards inflammatory or wound healing phenotypes[1-5]. Several approaches comprise the physicochemical properties of biomaterial devices, such as size, shape, topography, and chemistry. Indeed, the topography is a very important property that is known to influence macrophage attachment and phenotype, providing opportunities for the modulation of the macrophage function[6]. In this work, we evaluated the impact of biomimetic surface topographies on macrophages acute inflammatory response. For that, we selected 4 different biological surfaces, namely *E. coli, S. epidermis*, L929 cells and the Eggshell membrane. These surface topographies were obtained by soft lithography being replicated in spin casting PCL membranes. The analysis of the expression of pro-inflammatory and anti-inflammatory markers were performed in THP-1-derived macrophages. The results revealed that smooth surfaces induced a pro-inflammatory response contrasting with the surfaces of L929 cells and the Eggshell membrane surface topography that are more prone to induce an anti-inflammatory response. Herein, we were able to demonstrate the significance of surfaces that mimic the topography of biological substrates to modulate inflammatory responses. Our data suggests that immune-modulatory surfaces can boost the efficacy of scaffolds used in tissue engineering strategies.

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LASER-BASED SUBTRACTIVE MANUFACTURING FOR TISSUE ENGINEERING

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Clinical translation of tissue engineering-based therapies is currently limited by the difficulty in inducing essential vascularisation for tissue viability after transplantation. Thick and metabolically demanding engineered tissues require a defined microvascular network to provide sufficient nutrient and gas exchange. Laser ablation has emerged as a promising technology to fabricate custom-made perfusable microfluidic channels that mimic capillary beds and aid the vascularization of tissue engineered constructs. In this work, we developed a multistep patterning method to precisely create hierarchical vascular trees using a commercial 355 nm laser ablation system. In order to design physiologically relevant capillary networks that consider tissue geometry, physical constraints, and structure stability, vascular trees were generated using a constrained constructive optimization-based method [1]. Vascular trees were generated using Accelerated Constrained Constructive Optimization as arterial/venous matched pairs meeting at simple anastomoses. Batch optimization was used to minimize a combination of network volume and pump work, with post-build bifurcation asymmetry correction. Inter- and intra-network collisions were resolved, including padding to ensure vessel spacing. Vessels were smoothed and new collisions resolved before export. Subsequently, a slicing and tiling algorithm was developed to bridge the gap between 3D CAD model and laser software specific formats. Also, an optimization of the working parameters of LASER manufacturing tools (e.g., velocity, beam intensity, z-step, etc.) was required to precisely reproduce the 3D CAD model within a diversity of low stiffness hydrogels. The resulting vascular trees can be used to obtain capillary beds for tissue engineering applications and the developed method can be adapted to a multitude of other applications exploiting transparent hydrogel scaffolds.

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DECELLULARIZED KIDNEY EXTRACELLULAR MATRIX AS A BIOCOMPATIBLE BIOMATERIAL FOR KIDNEY REGENERATION.

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Kidney's function surpasses the filtration of toxins, and this organ presents a key role in the organism homeostasis. Isolated insults result in acute kidney injury (AKI). Repeated insults deplete the regenerative pool of kidney cells, progressing into an irreversible stage of renal failure. Dialysis is lifesaving for patients, but it doesn't prevent disease progressing and place high burdens in patients and health economy. The increasing number of patients in end-stage renal disease created an unprecedented organ shortage with the transplant waiting list increasing every year, tendency that is expected to be maintained in the next decades also as a consequence of COVID-19 pandemic.[1,2] Innovative therapies to restitute renal function, avoiding disease progression and the development of comorbidities are currently being explored. We hypothesized that decellularized kidney extracellular matrix (DKECM), processed into a hydrogel can be used as an injectable, biocompatible and biomimetic advanced therapy. We propose a therapy that will not only integrate into the host tissue, but also prevent renal fibrosis and organ atrophy. For that purpose, this work is focused on the direct exploration of the variables known to affect the host response, trying to maximize the *in vivo* efficacy. The effectiveness of the production under sterile conditions was assessed, with negative cultures for bacteria and fungi. The presence of residual detergents after the decellularization process was not detected by methylene blue assay. Preliminary results revealed the absence of the immunogenic epitope, alpha-galactosyl. The immunogenic potential of DKECM-based hydrogel will be further studied by the *in vitro* induction of an inflammatory response. Macrophage polarization will be explored and the presence of anti- or pro-inflammatory macrophages will allow a better prediction of *in vivo* inflammatory response. After these validations we expect to minimize the immune reaction upon implantation *in vivo*.

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HYDROGELS BASED ON CATECHOL-MODIFIED HYALURONIC ACID COMBINED WITH GRAPHENE DERIVATIVES FOR BIOMEDICAL APPLICATIONS

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Hydrogels are three-dimensional polymeric networks that are capable of absorbing and retaining a high amount of water while maintaining their structure due to chemical or physical cross-linking of individual polymer chains. The capacity of hydrogels to mimic the water uptake of the native tissues and promote the exchange of oxygen and nutrients makes them an ideal biomaterial for tissue engineering. So far, numerous natural and synthetic polymers have been proposed for the fabrication of hydrogels. Among them, hyaluronic acid (HA) has been considered as a very appealing candidate. HA is a natural component of the extracellular matrix and exhibits good biocompatibility, biodegradability, hydrophilicity, low immunogenicity, and unique viscoelasticity. Moreover, it can mediate cellular signalling and facilitate cell migration and proliferation, thus promoting tissue regeneration. However, HA-based hydrogels suffer from limitations such as weak bonding strength on wet biological tissue and poor mechanical and electrical performances. Therefore, it is of great significance to develop natural polymer-based adhesive hydrogels with fast-forming and high adhesion strength, along with enhanced mechanical and electrical properties. In nature, the mussel adhesive proteins have a unique ability to firmly adhere to different surfaces in aqueous environments via the special amino acid, 3,4-dihydroxyphenylalanine (DOPA). The catechol moiety in DOPA is a key group for adhesive proteins, which is highly informative for the biomedical domain. In fact, catechol contributes both to intermolecular crosslinking and to strong interfacial binding to organic/inorganic surfaces. Thus, inspired by this strategy, it is possible to formulate biomedical adhesive-hydrogels by conjugating the catechol moiety to polymeric chains. Furthermore, it has been shown that the reinforcement of polymeric matrices with nanoparticles, such as carbon-based nanomaterials, can enhance the performance of the final composites, by synergistically combine the strengths of the different components. Comparing to other carbon materials, graphene is potentially interesting to produce functional nanocomposites due to its excellent mechanical and electrical properties, as well as its high surface-to-volume ratio, which enhances the interface between filler and matrix, and enables to reach superior composite properties at low reinforcement loads. Since the investigation of composite hydrogels based on catechol-modified HA grafted with graphene derivatives is still incipient, this work aims the development and characterization of a bioadhesive hydrogel with enhanced mechanical and electrical properties for tissue engineering applications.

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DEVELOPMENT OF 3D LSPR (LOCALIZED SURFACE PLASMON RESONANCE) SENSORS BASED ON "SPONGY-LIKE" GELLAN GUM HYDROGELS (GG-SLH) FOR RAPID AND SENSITIVE DETECTION OF PROTEIN BIOMARKERS

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Protein biomarkers are fundamental for the diagnostic of high mortality conditions such as cancer. Localized surface plasmon resonance (LSPR) is the collective oscillation of electrons confined in metallic nanoparticles. The binding of biomarkers to the metallic nanoparticles causes a resonance peak shift upon the refractive index change, which translates into a maximum absorbance peak shift. These peak variations can be used and explored for the detection of biomolecules or liquids with different refractive indices. Commonly, LSPR platforms are highly miniaturized using a variety of substrates with thin layers of nanoparticles (NPs), in order to reduce reagents, use small samples, and reduce costs, however, these platforms also demand sensitive and customized detectors which hinders Point-of-Care (POC) applications and commercialization. We present a novel 3D LSPR platform base on gellan gum "spongy-like hydrogels" (GG-SLH) with integrated gold NPs (AuNPs). AuNPs are frequently used in LSPR biosensors due to their biocompatibility and simple bio-receptor immobilization. GG-SLH were engineered with >90% porosity, uniform pore sizes, and full recovery after pre-load of 0.1 N and tested up to 60% of strain. These excellent mechanical properties are essential for a biosensing platform since it enables manipulation, microfluidic integration, and multistep assays. AuNPs can be integrated into GG-SLH maintaining their high transparency when reconstituted with solutions. This property enables the LSPR detection of AuNPs local environment in a 3D highly porous structure within the millimeter scale. This feature enables LSPR measurements using a standard microplate reader due to increase sensitivity caused by a higher amount of AuNPs in a specific area, without compromising analyte/solvent diffusion due to the platform's high porosity. Results have shown that AuNPs-GG-SLH can detect variations in medium refractive index within 30 min when changing PBS aqueous solutions to different concentrations of glycerol and ethanol using a standard microplate reader. Also, it was observed that the platform can be tuned to different ranges of detection by modifying the quantity of AuNPs and thickness of the GG-SLH. GG-SLH with integrated AuNPs have great potential as LSPR platforms for POC applications since they allow rapid and sensitive detection with a cost-effective and standard microplate reader. Future work will consist in the functionalization of AuNPs with antibodies and specific detection of cancers antigens for cancer diagnosis using standard detectors for LSPR label-free techniques.

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CRYOPRESERVED HUMAN ADIPOSE TISSUE-DERIVED STROMAL VASCULAR FRACTION FOR THE GROWTH FACTOR-FREE VASCULARIZATION OF BLUE SHARK COLLAGEN SPONGES

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Vascularization is a critical aspect of every tissue engineering (TE) approach, especially in 3D constructs. The formation of a network of capillaries is necessary to ensure adequate delivery of nutrients and oxygen to cells within the constructs, as well as fast anastomosis with the surrounding tissue. Pre-vascularization of these constructs before implantation can be a solution. However, cell sourcing is a limiting issue. Adipose tissue is regarded as a privileged source of mesenchymal progenitor cells due to its easy accessibility and abundancy. This tissue hosts mature adipocytes, as well as a stromal vascular fraction (SVF) which comprises, in addition to those mesenchymal progenitors, several other cell types including fibroblasts, pre-adipocytes, endothelial progenitors, endothelial cells and hematopoietic cells. Due to this composition, the SVF of adipose tissue is highly angiogenic has allowed the growth factor-free vascularization of TE constructs. Although collagen from mammalian sources is widely used to produce TE constructs, regulatory issues associated with the risk of disease transmission have boosted the search for new collagen sources such as from marine organisms. Collagen from otherwise wasted blue shark skin is easily obtained and has shown similar features to that of mammals. In this work, a pre-vascularized blue shark skin collagen sponge was created by using cryopreserved SVF from human adipose tissue in an extrinsic growth factor-free manner. Sponges' pre-vascularization was assessed *in vitro* by immunohistochemistry and their functionality was tested *in vivo* using a chick chorioallantoic membrane (CAM) assay. After 7 days of *in vitro* culture, CD31 expression pattern demonstrated the formation of a vessel like network. After 4 days of *in ovo* implantation, chick lectin staining and vessel density quantification demonstrated an increase in vessel recruitment in pre-vascularized sponges when comparing with sponges without SVF. CD31 expression pattern demonstrated the integration of the pre-vascular network in the CAM and *in situ* hybridization confirmed the presence of the seeded human cells. These results demonstrate the potential of cryopreserved SVF to assist in the vascularization of TE constructs in an extrinsic growth factor-free manner, allowing a simplified and cost-efficient methodology to ensure construct integration after implantation.

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GELLAN GUM-BASED HYDROGELS BIOFUNCTIONALIZED WITH LAMININ-DERIVED PEPTIDES FOR MYOCYTE DIFFERENTIATION AND ALIGNMENT

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Recent advances in skeletal muscle tissue engineering have revealed the importance of simulating the extracellular matrix (ECM) while engineering new tissue to trigger cellular maturation. Herein, we propose the tethering of laminin-derived peptides (CIKVAVS (V), KNRLTIELEVRTC (T), and RKRLQVQLSIRTC (Q)) into nature-based gellan gum (GG) hydrogels to reinforce their adhesive traits. First, the binding of murine skeletal muscle cells (c2c12) to each peptide was quantified. Then, each peptide was conjugated to the backbone of divinyl sulfone (DVS) functionalized GG and the conjugation efficiency determined. GG-based hydrogels were prepared in four gradual concentrations and characterized by bio-AFM. C2c12 were seeded on top of the hydrogels, and the cellular adhesion, spreading and differentiation was monitored along 21 days through phalloidin and Myosin Heavy Chain staining. Finally, cell alignment was promoted with a 100 µm micro-grooved substrate. The maximum percentage of cell binding to peptide was 9.53%, 47.97%, and 25.77% for V, T, and Q peptides respectively. The efficiency of peptide conjugation to GGDVS was 60.21%, 39.54, and 31.04, respectively for V, T, and Q peptides. In general, higher modulus values were in line with higher polymer concentration except for V peptide, which had the same stiffness pattern for all concentrations. Cellular spreading, differentiation, and alignment was promoted in peptide Q-biofunctionalized hydrogels and preferably in stiffer gels. Overall, biofunctionalized GG-based hydrogels are reliable biomaterials for providing the cells with a mimicry microenvironment for cellular differentiation. They serve as a potential epi-muscular patch to be directly applied onto the damaged muscles.

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INJECTABLE THERMOSENSITIVE CATECHOL-MODIFIED CHITOSAN HYDROGELS WITH SUPERIOR ADHESION FOR BONE TISSUE REGENERATION

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Chitosan is one of the most abundant polysaccharides of natural origin, mainly obtained from crustaceans, which is widely used in several biomedical applications including bone tissue engineering due to its biocompatibility, biodegradability, promoting cell growth and osteoconduction properties.[1] Chitosan can be prepared as hydrogels following heat application, making it suitable as an injectable thermogel.[1] Chitosan hydrogel can be produced through electrostatic interactions, using b-GP, an organic compound found in the body, already approved by FDA for intravenous administration. It also has been used as a catalyst to cause a sol–gel transition in Chitosan solutions at physiological pH and temperature. When adding b-GP, a weak base, to Chitosan aqueous solutions, the polymer remains in solution at neutral pH and room temperature, while homogeneous gelation of this system can be triggered upon heating. The introduction of ß-GP with sol-gel transition will increase the pH into the physiological range and will allow for controlled gel formation at 37ºC.[2] The main challenge of these hydrogels is the lack of bioadhesion between the hydrogel and tissue i.e. the capacity to bind the target tissue and the low cell affinity/adhesion.[3] The marine mussels proteins have been inspired to create one of the most popular biomimetic that adhere even in wet environments, in particular, 3,4-dihydroxyphenyl-L-alanine that contains catechol groups, responsible for the strong adhesion.[4] The bioadhesion is an advantage for any material for tissue engineering application since it prevents the detachment of the biological tissue and is capable to wound healing through the reconnection of broken bonds, endure high stretchability, and reduce the risk of cracking.[5] This work aims the production of a new class of adhesive hydrogels based on a very known system of Chitosan/ß-GP hydrogels for bone tissue regeneration applications.

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MARINE GELATINE METHACRYLOYL-BASED HYDROGELS FOR TISSUE ENGINEERING

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Materials from marine organisms have a wide range of characteristics that may justify their potential application in the biomedical field, assuring the sustainable exploitation of natural resources and the valorization of by-products. In particular, marine gelatine is free of transmissible diseases (a non-negligible risk associated with the use of mammalian resources) and enable high production at low cost.[1] In this study, gelatine was extracted from halibut skins and further functionalized with methacrylate groups by reaction with methacrylic anhydride to develop the photosensible gelatine methacryloyl (GelMA).[2,3] GelMA was used to prepare photocrosslinked hydrogels through UV light and a photoinitiator. To improve the biological performance of the envisaged hydrogels, GelMA was combined with glycosaminoglycans (GAGs) such as hyaluronic acid (HA) and chondroitin sulfate (CS). HA has a high capacity for lubrication, adsorption and water retention, while CS has resistance to compression. GAGs methacrylation reaction was deemed necessary, rendering methacrylated hyaluronic acid (HAMA) and methacrylated chondroitin sulfate (CSMA),[4] properly characterized by FTIR and NMR to assess the effectiveness of chemical functionalization. Three different concentrations of GelMA were combined with different ratios of CSMA and HAMA to produce biomechanically stable hydrogels upon photocrosslinking. In order to characterize the produced hydrogels, swelling, enzymatic degradation and rheology tests were performed, and it's concluded that the formulation with 20% GelMA and HAMA/CSMA mixture presents the best results. The marine GelMA-based hydrogels produced in this work are being tested as a matrix for a chondrocyte culture for cartilage tissue engineering.

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TUNING INFLAMMATION AND HYPERTROPHY IN ADIPOGENIC CELL SHEETS TO MIMIC OBESITY IN VITRO

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The worldwide epidemic of obesity has motivated studies aiming at characterizing the mechanisms regulating obesity-linked adipogenic and inflammatory responses using *in vivo* and *in vitro* models. One of the main features of adipose tissue is its ability to expand to accommodate energy surplus. In obesity, adipose tissue becomes severely dysfunctional failing to appropriately expand. This unhealthy expansion of adipose tissue is mainly driven by hypertrophic processes accompanied by a shift to a pro-inflammatory adipokine and cytokine profile. Current *in* vitro obesity research has focused on adipose tissue models that rely on two-dimensional cell culture that fails at recapitulating the three-dimensional (3D) microenvironment of the *in vivo system*. In this sense, this work aims at developing 3D adipose-like tissue analogues bearing hypertrophied adipocytes within an inflammation milieu. For that, human adipose-derived stem cells were seeded at sub-confluency for 3 days with ascorbic acid supplementation and then cultured for 6 days in adipogenic differentiation medium plus 9 days in adipogenic maintenance medium[1]. The generated cell sheets were then submitted to different time combinations of TNF-α (100 ng/mL), an inflammatory cytokine, and palmitic acid (PA) (0.5 mM), a saturated fatty acid that promotes adipocytes' enlargement, for further 13 days. Adipocyte size, lipid accumulation and glycerol release were assessed as adipogenic indicators, together with the secretion of inflammatory cytokines (TNF-α and IL-6). The combination of hypertrophic and inflammatory stimuli for 13 days (PA+TNF-α), and the treatment with PA alone for 10 days followed by a combination of PA+TNF-α for 3 days were the ones that led to a higher TNF-α and IL-6 secretion, accompanied by larger adipocytes and increased glycerol release. Based on these results, single layer adipogenic cell sheets were stacked to produce 2 and 3-layered constructs which were then submitted to the previous optimized treatments. The preliminary assessment confirmed that there is a tendency for increased secretion of both TNF-α and IL-6 together with increased adipocyte size. These results showed that we were able to develop a 3D adipose-like tissue analogues characterised by enlarged adipocytes with a pro-inflammatory profile thereby resembling the characteristics of the obese adipose tissue.

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NATURAL NANO-TEXTURED BACTERICIDAL SURFACES IN INSECT WINGS: A WIDE-BREADTH SCREENING

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Antibiotics are one of the best current medical tools to fight disease, preventing countless deaths since their discovery in the 19th century. However, due to their misuse and abuse and despite new discoveries every year, antibiotics are progressively becoming less and less effective – the result of the unstoppable evolution of the target bacteria. As such, there is great need for technology that allows us to combat bacteria without depending solely on these compounds and prevent the development and evolution of multi-resistant bacterial strains. This is of special importance in a medical environment where the cleaning and disinfection of surfaces is of crucial for stopping the spread of infection. Nature is often a great source of inspiration for science and remains true in this case, by serendipitous chance. In recent years, while analyzing the optoelectronic properties of cicada wings it was found that they have covered in a very defined nano-texture that, among other interesting and useful properties (such as hydrophobicity and self-cleaning), can kill bacteria on contact, relying on the physical structure and their physical interaction with bacteria alone. In the case of cicada wings, this is achieved by the array of nanopillars present on the wing surface that have the ability to disrupt or puncture the bacterial membrane. Since this discovery there have been identified only a few more insects that possess nano-textures with bactericidal power, such as damselflies and dragonflies. Our objective with this work is to further delve into the potential wealth of nano-textures present in the vast group of insect species that exist and identify the more suitable ones to be synthetically replicated later on for practical applicability. Besides the pure scientific importance for the field of Biology of identifying these never before described features, searching naturally occurring nano-textures allows us to take advantage of evolutionary processes and subsequent naturally occurring optimization to immediately reach effective structures and patterns. So far, more than 140 distinct winged insect species from around the world have had their wings and potential nano-textures analyzed and characterized by High Resolution Scanning Electron Microscopy. Several novel nano-textures and insights have been discovered, with the orders showing the most potential being Diptera, Odonata and Hemiptera. Microbiology procedures are currently underway to quantify the bactericidal power of these structures and replicate them in both inorganic and organic substrates, such as polymers, ceramics and metals.

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STRUCTURE AND COMPOSITION OF THE CUTICLE AND CALCIFIED PARTS OF AN ATYPICAL CRUSTACEAN - THE GOOSE BARNACLE POLLICIPES POLLICIPES

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Arthropods are the largest animal phylum, including insects, spiders and crustaceans, and are characterized by their body covered primarily of chitin. In the biomedical field, this biopolymer has been isolated from marine crustaceans, mostly from shrimps and crabs' cuticles resulting from seafood industry, to produce chitosan. Some crustacean taxa exhibit a variety of body shapes and adaptations to particular habitats and environmental conditions being relevant to broaden the knowledge of this biopolymer to other crustacean protective coverings. The barnacle Pollicipes pollicipes is an atypical sessile crustacean characterized by their body parts covered with calcified plates and a peduncle attached to a substrate and which is covered with a cuticle. In this work the composition and structure of their covering parts were characterized. Imaging of the tergum plate revealed a compact homogeneous structure of calcium carbonate, a composition common among marine invertebrate hard structures[1]. The cuticle consists of an internal homogenous zone of successive layers made of sheets parallel to the surface and an outer zone which is denser and covered with scales, with a similar composition to the tergum plate, and arranged parallel and oriented semi-vertically. Structural and biochemical characterization confirmed a bulk composition of α-chitin and suggest the presence of collagen and other structural proteins (e.g.elastin[2]). Young modulus of the cuticle occurred within the range of values described in elastomers[3]. The removal of calcified components exposed rounded holes and detailed the structure of the lamina. It also affected their mechanical properties by increasing significantly the Young Modulus. With the deproteinization, changes were related to cuticle shrinkage. This structural arrangement may confer elasticity to the material to deal with environment variability (e.g.wave action) and at the same time protection against predation. This soft and flexible cuticle, comparable to the molting crustaceans 'cuticles, may provide bioinspiration for the development of biocompatible and mechanical suitable biomaterials for wound healing.

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A STATISTICAL DESIGN OF EXPERIMENTS (DOE) APPLIED TO TISSUE ENGINEERING SCAFFOLDING MANUFACTURING BY COMBINATION OF MARINE-ORIGIN BIOPOLYMERS

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In the last decade, marine-derived polymers have been considered a natural alternative to mammal sources to provide, in a sustainable form, valuable bioactive compounds for the biomedical sector, as tissue engineering scaffolding applications. Mainly, these compounds have similarities with proteins and polysaccharides present in the human extracellular matrix (ECM) and can be considered safe due to their low risks associated with zoonosis and overcoming social/religious-related constraints. However, the manufacturing process of scaffolds (such as cryogels) with biomimetic properties is quite challenging due to ECM complexity and diverse process variations (as temperature, polymer concentration, sources, among others) that influence the scaffolds manufacturing. In fact, little is known about the influence of these parameters and their interactions. To overcome these difficulties, factorial design of experiments (DoE) and response surface methodology (RSM) emerge as a friendly statistical tool to determine the most influential parameter and optimize the processes. In this work, we hypothesized that a design of experiments (DoE) model applied on the Box–Behnken design (3 factors and 3 levels) could optimize the collagen-chitosan-fucoidan cryogels manufacturing. The responses selected to measure the DoE were rheological oscillatory measurements, antioxidant concentration, and adenosine triphosphate (ATP) concentration. This data showed that fucoidan concentration and temperature significantly influenced cryogel formation, creating a stable internal polymeric network promoted essentially by ionic cross-linking bonds. Overall, the proposed DoE model was considered suitable for predicting the best parameter combinations needed to develop these cryogels, being attributed the best condition to gels produced at -80 ºC and composed of 5 % of collagen, 3 % of chitosan, and 10 % fucoidan.

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ECO-FRIENDLY PRODUCTION OF 3D-PRINTED SCAFFOLDS FROM COD FISH PROCESSING BY-PRODUCTS FOR BONE TISSUE ENGINEERING

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The ability to manage the enormous amounts of waste produced by the food processing industry is a challenging problem. With the rapid growth of the fishing industry, a remarkable amount of fish processing by-products is generated every year, and about 40–50% of its total weight is discharged as waste during the process. However, fishbones and skins have a large number of bioactive compounds, such as calcium phosphates (CaPs) and collagen, which can serve as a raw material to be applied in health-related investigation. Recently, marine collagen use has gained more attention because of its low risk of disease transmission to humans, no related religious constraints, and fewer regulatory and control problems. Collagen has also been widely used in bone tissue engineering, since it offers advantages, like the presence of biochemical cues, that support cell attachment, proliferation, migration, and differentiation. At the same time, CaPs-based materials have shown outstanding osteoconductivity/osteoinductivity in bone regeneration. Since the combination of synthetic and natural-origin materials arises as one of the best solutions to obtain 3D scaffolds for the regeneration of bone tissues, in this study, a biodegradable synthetic polymer - polycaprolactone (PCL) was combined with calcium phosphate produced from fish bones to develop a scaffold by 3D printing, further functionalized by incorporation of collagen to improve cytocompatibility and performance. The fishbone powder size and morphology were proven determinants in their uniform distribution along the produced filament, which was used for the 3D printing of porous structures. To modify the hydrophobicity inherent to PCL scaffolds, a superficial treatment was done with NaOH, allowing the scaffolds to be coated with marine collagen. The produced composite scaffolds presented regular and reproducible structures, and after being evaluated from chemical and physical perspective, it was possible to conclude that the combination of PCL and fishbone powders 3D printed scaffolds with marine collagen functionalization created a hierarchical material with a potential application in bone tissue engineering.

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SUPERCRITICAL EXTRACTION OF ECM COMPONENTS FROM CELL SHEETS

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Supercritical carbon dioxide (scCO2) technology has been recently used in the field of TERM as a method of processing biomaterials. Additionally, this technology has been also applied to decellularize tissues such as aorta, myocardium, pericardium, cornea and adipose tissue. Its great advantage relies on the fact that scCO2, with a critical point at mild conditions (31.1°C and 7.38MPa), is non-toxic, non-flammable, relatively inert and can be removed easily by depressurization. Despite this, scCO2 is apolar and the addition of an entrainer is required to eliminate charged molecules such as phospholipids. CO2-philic detergents are based on detergent alcohols (fatty alcohols with carbon chain length in the range between of C12-C18), rendering these molecules with surfactant-like properties, as well as potential to interact and diffuse with supercritical CO2. Recently, a CO2-philic detergent, Dehypon, was used as entrainer to decellularize articular cartilage, tendon and skin. Based on this, we propose the use of a combination of Dehypon/CO2 as a supercritical fluid with higher potential to decellularize cell sheets. We hypothesised that the use of Dehypon/CO2 will allow using less harmful conditions than those used in traditional detergent-based decellularization methods thus attaining a higher level of preservation of extracellular matrix (ECM) components. Human dermal fibroblasts (hDFb) and adipose stem cells (ASC) cell sheets were prepared as previously reported (M. T. Cerqueira et al., 2013; M. T. Cerqueira et al., 2014). For decellularization, cell sheets were incubated with 0.1% Triton-X100 and 20 mM NH4OH followed by another incubation with DNase or with 0.2% Dehypon and scCO2. DAPI staining validated the disruption of cell nuclei and subsequent DNA removal from the cell sheets, while preserving the overall matrix ultrastructure, independently of the method used. Total protein quantification using the Bradford assay showed a lower loss of protein when scCO2 technology was used. Importantly the SDS-PAGE electrophoresis confirmed maintenance of the native cell sheet profile and the absence of protein degradation using the scCO2 technology. Overall, the use of the use of Dehypon/CO2 and the scCO2 technology allowed an improved preservation the cell sheet's ECM which will allow extending the use of these cell-derived extracts to develop cell-specific tissue models.

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MICROALGAE EXTRACTS AS NATURAL SOURCE OF BIOACTIVE INGREDIENTS FOR ANTIAGING COSMETICS

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The pursuit for antiaging cosmetics with natural bioactive ingredients has increased in recent years. Microalgae, due to their pigments, peptides, fatty acids and polysaccharides content, present anti-inflammatory, antioxidant and antiaging properties with great cosmeceutical interest [1]. Thus, in this study, we aimed evaluating the antiaging properties of microalgae extracts from 5 selected strains by analyzing their UV light protection and skin rejuvenation potential. Microalgae biomass was extracted using 4 solvents (water, ethanol (48% and 96%) in water and acetone) at different pressure/time conditions (300-500 MPa/9-15 min) at room temperature. The cytotoxicity of the extracts (500, 100 and 10 µg/ml) was evaluated in human skin-derived cells, i.e., fibroblasts, keratinocytes and melanocytes, using the MTS assay. Photoprotection was evaluated after assessing the metabolic activity of keratinocytes and melanocytes after exposure to extracts and UV light. Skin rejuvenation was evaluated through the analysis of fibroblasts proliferation and extracellular matrix deposition (elastin, collagen, and glycosaminoglycans) and remodeling (MMP1). IC50 varied between 100 and >500 µg/ml among the different microalgae extracts and cell types. Regarding the UV light photoprotection, *Chlorella bio* extracts presented lower phototoxicity. Regarding the skin rejuvenation, microalgae extracts did not affect the production of elastin (150-400µg/µg of DNA), collagen (50-200µg/µg of DNA) and glycosaminoglycans (5-20 µg/µg of DNA), although MMP1 increased. Additionally, *Nannochloropsis oceanica* and *Tetraselmis chuii* extracts increased the metabolic activity and proliferation of fibroblasts. Overall, microalgae extracts are rich in phytochemical components and are safe to use in skin cells at specific concentrations. Also, a potential of these extracts to increase cell renewal was detected.

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POTENTIAL OF ATLANTIC CODFISH (GADUS MORHUA) SKIN COLLAGEN AND DERIVATIVES ON SKINCARE

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Marine organisms have been arousing interest to the biomedical and cosmeceutical industry, as an alternative source of collagen[1,2]. The Atlantic codfish (*Gadus morhua*) is a relevant species for this matter, since it is processed in large quantities by the food industry, rendering an important amount of by-products that can be valorized as source of valuable compounds as collagen[1]. In the present work, collagen from Atlantic codfish skin was extracted, characterized and used to develop membranes, evaluating its potential for skincare applications. The collagen was extracted from two different skin batches, using acetic acid (ASColl)[1], resulting in yields between 2.9% and 4.8% (w/w). The results from SDS-PAGE, FTIR and XRD analyses indicated the preservation of the collagen native structure and chemical composition, without significant differences, with profiles similar to the one observed for commercial bovine skin collagen, used as reference. Also, the assessment of the metabolic activity of skin fibroblasts (BJ cell line), exposed to the collagen extracts with concentrations up to 10 mg/mL, revealed no cytotoxicity. The collagen membranes were produced using a 1% collagen solution in acetic acid, and two crosslinking methods were tested, using HMDI or EDC/NHS solutions[2,3]. The reaction of collagen with EDC/NHS resulted in a non-detachable and easily degradable membrane, while non-crosslinked collagen and the collagen-HMDI solution resulted into mechanically stable and detachable membranes. According to SEM analysis, these membranes did not present significative differences between batches or conditions, showing a smooth surface. Regarding the biodegradation test results, using collagenase, there are no significative differences between conditions, questioning the crosslinking efficiency. Considering the water uptake, non-crosslinked membranes have higher rates, comparatively with crosslinked ones, with both showing a hydrophilic feature (water contact angle < 90°). The effect of the developed collagen membranes on skin fibroblasts and keratinocytes activity is currently being assessed to evaluate their biomedical and cosmeceutical potential.

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COLLAGEN EXTRACTION, ISOLATION AND CHARACTERIZATION FROM MARINE SPONGES

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Collagen has mainly a structural function and is among the most abundant groups of proteins in vertebrate as well as in invertebrate species. For those reasons, it has become widely used in tissue engineering and regeneration approaches. Moreover, collagen has unique properties such as high biocompatibility, high biodegradability, low antigenicity and the presence of biochemical cues for cell adhesion and growth [1]. At the industrial level, collagen from bovine and porcine origin has been the most commonly used. More recently, the use of collagen from these sources has become a matter of concern, due to limitations imposed by religious and social constraints, as well as by some zoonotic diseases outbreaks, such as BSE or the swine flu, which posed high pathological risk [2]. Therefore, marine organisms arose as promising alternative collagen sources, gained wide acceptability and became highly attractive to the industry. Marine invertebrates compose a significant fraction of the aquatic biodiversity and have potential to provide several materials with interest in biotechnological applications, such as sponge-derived collagen [3]. This work aims at extracting and isolating pure collagen from marine sponges occurring in the northern coast of Portugal, with the focus on its potential application as a high added value biomaterial for tissue engineering purposes. In order to achieve that goal, marine-based collagen is being extracted from sponges according to the method of Matsumura (1974) [4], based on an alkaline procedure for the extraction of non-acid soluble collagens. The biochemical and structural characterization of the isolated collagens is being carried out to evaluate their potential use on biomaterials. The knowledge produced will also be relevant for the biotechnological valorization of marine biomass from the north Atlantic, based on sustainable valorization approaches.

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CHARACTERIZATION OF CODFISH GELATIN: A COMPARATIVE STUDY OF FRESH AND SALTED SKINS AND DIFFERENT EXTRACTION METHODS

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Gelatin is generally known as the product derived from thermal denaturation of collagen given origin to an unfolding and cleavage of the triple helix resulting in a polydisperse mixture of polymers [1]. Due to the similarities with collagen, it has been widely used in tissue engineering applications to produce new biomaterials or as cellular carrier [1,2]. The primary sources of gelatins are from mammal origin, yet by-products of the fishery industry have also been considered as raw materials for gelatin production [3]. Different extraction processes are described in literature to obtain gelatins from fish skins, however there is a lack of information about the preservation of those skins (fresh, frozen, salted, etc.) and how that affects the final yield and properties of gelatin. In this study we present a comparative analysis between gelatins produced from fresh and salted codfish (*Gadus morhua*) skins using different extraction protocols based on methodologies described in literature. The characterization of the materials was assessed based on yield of extraction, amino acid composition, molecular weight distribution, rheological properties, and gel strength, as well as the cell compatibility of the gelatins envisaging future biomedical application. Methodologies using sulphuric + citric acid during the washing processes and a thermal extraction in water demonstrated to produce gelatins rich in OH-proline + proline, enhanced gel strength and rheological behavior, and with higher melting points. Differences between fresh and salted skins were found, with gelatins derived from salted skins demonstrating lower viscoelastic properties, as well as gel strength, when compared with gelatins from fresh skins. Our research has highlighted the importance of choosing the right protocol according to the final application of the gelatins, as a tool on the quest for the sustainable valorization of fish by-products, included in a blue and circular bioeconomy framework.

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3D PRINTED SCAFFOLDS BASED ON MARINE SPONGE COLLAGEN AND SR-DOPED FISH BONES FOR BONE TISSUE ENGINEERING

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Collagen is the most abundant structural protein, being extremely similar in both vertebrates and invertebrates. Currently, the main sources of collagen are bovine and porcine by-products, but risks of zoonosis and religious/social constraints have encouraged research for alternative sources, namely for biomedical application [1]. *Chondrosia reniformis* is a marine sponge being studied as a potential sustainable source of collagen known to be more glycosylated than other collagens in Metazoa [2]. The high abundance of glycosaminoglycans favours cell attachment and proliferation, enhancing its regenerative potential as biomaterial. Additive Manufacturing (AM) is arising as a versatile and potent tool for production of biomaterials, in particular tissue engineering scaffolds by 3D printing. However, its widespread use is still hampered by the limited printable materials. The aim of this work was to develop new bioinspired and bioresorbable inks for AM, composed of *C. reniformis* collagen and ionic-doped fish bones (FB), as building blocks for the production of scaffolds towards bone regeneration. Alginate (Alg) was used as model biopolymer for implementation of the printing methodology given its easily ionic induced jellification. Since the use of collagen materials require the establishment of adequate crosslinking procedures and/or polymer blending, collagen/alginate blendings were tested. In this regard, we aimed to implement 3D printing methodology of *C. reniformis* collagen combined with alginate and strontium-doped FB (MColl+ Alg+FB(Sr)). The preparation of ionic-doped FB nanopowders was based in isolation of calcium phosphates from codfish bones by calcination and further Sr doping, knowing its impending roles in biological processes. Three formulations were tested and compared: Alg+FB, MColl+Alg+FB and MColl+Alg+FB(Sr). To this end, the scaffolds' physicochemical characteristics and the cellular responses were studied by *in vitro* cell culture experiments, assessing cytocompatibility to Saos-2 cell line. It was concluded that MColl+Alg+FB(Sr) 3D printed scaffolds presented the best results, as they strongly promoted Saos-2 survival and proliferation. It was demonstrated that *C. reniformis* collagen and FB(Sr) are beneficial for biomedical applications, being a promising alternative towards bone regeneration therapies.

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STRUCTURAL AND MECHANICAL DIFFERENCES BETWEEN CODFISH SKIN AND SWIM BLADDER COLLAGEN.

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Fish collagen is being growingly studied as alternative to mammal collagen in different areas of application, from food to medicine [1]. This study aimed to analyze the properties of codfish collagens, considering different codfish by-products – skins and swim bladders – and methods, envisaging biomedical applications. Collagen was extracted from cod skin and swim bladders using acetic acid (ASColl and Collsb, respectively) and from cod skin using CO_2 acidified water (AWCs), with extraction yields of 4± 0.68 %, 3.06±1.51 % and 12.28±2.17 % for ASColl, Collsb and AWCs, respectively. The SDS-PAGE profiles obtained are compatible with type I collagen, exhibiting the characteristic bands (β, α1 and α2). Moreover, the collagen extracts had identical profiles when analyzed by XRD, UV-Vis and FTIR-ATR suggesting that they present similar structures and chemical compositions. The presence of elemental impurities was also tested, with all samples presenting levels below the regulatory limits. Furthermore, the rheological tests shown collagen solutions as non-Newtonian fluids exhibiting a typical shear thinning behaviour, with the gel character of the collagen solutions decreasing with increasing temperature and became stiffer with increasing concentration. Among the evaluated samples, Collsb exhibited higher mechanical properties, suggesting more cohesive materials and potential for the development of injectable biomaterials. This will be further explored with cell compatibility tests, while deepening the biochemical characterization to understand the different mechanical performance.

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