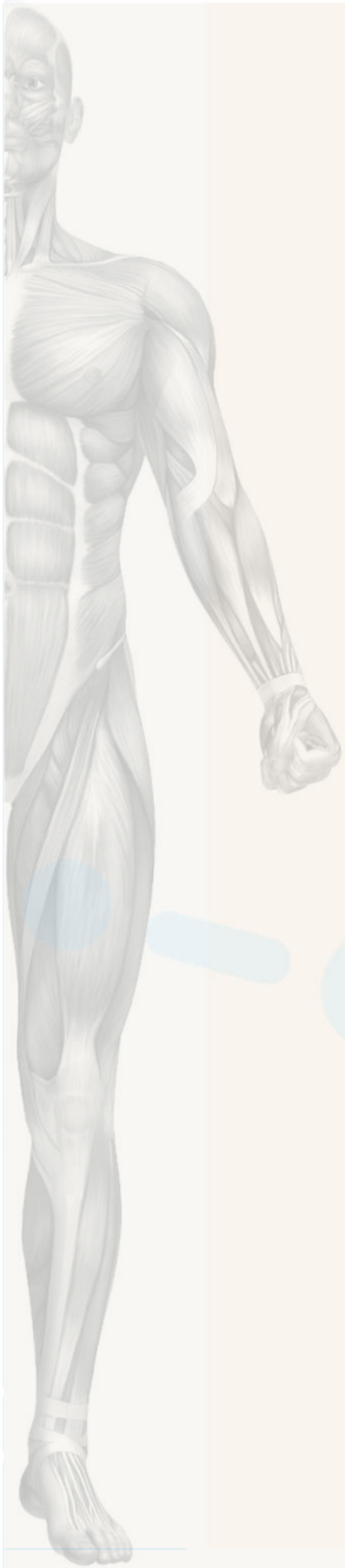
3rd WORKSHOP



GENE & CELL THERAPY AND CLINICAL APPLICATIONS

25 - 27 October

Porto - Portugal

ACHILLES

ADVANCING TENDON REGENERATIVE THERAPIES

ACHILLES®

ADVANCING TENDON REGENERATIVE THERAPIES

MESSAGE FROM THE CHAIRS

The **Third Achilles Workshop** is being organized in the scope of the European Horizon 2020 Twinning project Achilles: Overcoming specific weaknesses in tendon biology to design advanced regenerative therapies (achilles.i3bs.eu), coordinated by the 3B's Research Group of the University of Minho (UMinho, Portugal), with the participation of The Regenerative, Modular & Developmental Engineering Laboratory, National University of Ireland Galway (Ireland), the Department of Trauma Surgery, University Regensburg Medical Centre (Germany).

In the framework of the Achilles project several methodological tools/activities (Thematic workshops, staff exchanges, etc) will be implemented focusing on uncovering the main mechanisms underlying tendon homeostasis, pathology and regeneration, through an integrated approach using knowledge from a multidisciplinary consortium in research areas including biomaterials and tissue engineering, cellular and molecular biology of disease mechanisms, biophysical strategies, as well as genetics and gene therapy, envisioning the development of advanced regenerative therapies for tendon diseases.

In the **Third Achilles Workshop,** we will be guided by the hand of distinguished speakers through ***Gene & Cell Therapy and Clinical Applications***. The event is organized to promote a close interaction between these highly skilled experts in the field with students and young researchers, through a number of different scientific and social activities. Moreover, the program will also include speakers on transversal topics such as ethics in research, intellectual property management and scientific writing, which will also allow students and young researchers to gain and train new skills. Therefore, we expect a fully engaged audience, vivid and eager to discuss their data and knowledge among peers, to share and to learn.

The workshop will be held in Fundação Dr. António Cupertino de Miranda (FACM) in the beautiful city of Porto, elected the 2017 European Best Destination. Porto is one of the oldest European centers and its historical core is a World Heritage Site since 1996. The city has been going through a renaissance in the last few years and is considered an emerging capital of food and fashion. Additionally, Porto is famous by the Port wine

cellars that can be found across the south Douro river shore. In summary, a wonderful city providing a unique atmosphere and character together with an outstanding perspective of history, food and wine.

Therefore, we hope that this workshop provides you a delightful scientific experience in this beautiful historical city of Porto!

The Workshop Chairs

Manuela Gomes

Rui L. Reis

Ana Gonçalves

Manuel Gomez-Florit

SUPPORT

The Achilles Workshop would like to sincerely thank the following support:

3B's

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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eTwinning

The ACHILLES project has received funding from the European Union's Horizon 2020 Research and Innovation programme, under the Grant Agreement Number 810850. This Proceeding Book reflects only the author's view and the Commission is not responsible for any use that may be made of the information it contains.

ACHILLES
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GENERAL INFORMATION

All the information contained in this book is accurate at the time of its publication. The Workshop Organizers reserve the right to make modifications.

WORKSHOP CHAIRS

Manuela E. Gomes
Rui L. Reis
Ana I. Gonçalves
Manuel Gomez-Florit

LOCAL ORGANIZING COMMITTEE

Adriana Vinhas
Alberto Pardo-Montero
Ana Almeida
Ana Guerra
Ana Luísa Graça
Luísa Rodrigues
Márcia Rodrigues
Margarida Miranda
Mahwish Syeda Bakht
Rui Domingues
Rosa Monteiro
Simão Teixeira

WORKSHOP PARTICIPATION & PRESENTATIONS

WORKSHOP VENUE

The workshop will be held in Fundação Dr. António Cupertino de Miranda (FACM) in Porto, Portugal, at the Auditorium II.

Getting to and from the venue

You can use public transportation (bus lines 502 and 504) or private transportation (taxi) to get to the congress venue.

Registration

You are automatically registered. Please address yourself to the Registration desk to collect your badge and documentations.

Opening hours

Monday, 25th October 9:30 – 18:30
Tuesday, 26th October 9:30 – 18:30
Wednesday, 27th October 9:30 – 12:30

Presentations

- The Keynote Lectures and Master Classes consist in 45-50 minutes presentations followed by 15-10 minutes discussion.

- The Transversal Skills Trainings consist in 30-45 minutes presentations followed by round table discussion.

- The Short Oral presentations consist in 8 minutes presentations followed by 2 minutes discussion.

E-Poster Area

The posters will be displayed in LCD monitors available at the entrance of the auditorium. Please check your poster code in the Posters List herein included.

Short Oral Competition

We have organized a Short Oral competition evaluated by the Keynote speakers, and the top scored presentation will be awarded.

Instructions

You can use your own computer or bring the presentation in a USB Memory stick; a computer will be available. If using the Workshop computer, PowerPoint presentation or PDF are allowed. Please note that you need to load it on the Workshop computer and check it at least 2 hours before the start of the session.

Certificate of Attendance

A Certificate of Attendance to the Third Achilles Workshop will be sent by e-mail to all registered participants after the Workshop.

Photography Policy

Recording and photographing the Third Achilles Workshop presentations will not be allowed.

Liability

The Organising Committee of the Workshop accepts no liability for participant personal injuries or loss/damage to personal property either during or as a result of the Workshop, 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 Workshop Organisers cannot accept any responsibility for personal accidents and damage to the private property.

SOCIAL EVENTS

Lunch and Get-together reception planned for the workshop are included in the registration. Lunch will be at the Restaurant of the FACM.

Get-together Reception

Day 1 – Monday, 25th October – 17:30 to 18:30
Foyer of the FACM
Dress Code: Smart Casual

Dietary Requirements

Please inform Organizing Secretariat by email or at registration desk as soon as possible in case you have any dietary requirements.

COVID-19 Requirements

According to the DGS (General Directorate of Health) and the venue's recommendations, all participants need to present the EU Digital COVID Certificate or, in alternative, proof of a test with a negative result for COVID-19 (PCR Test within 72 hours prior to the events or Antigen Test within 48 hours prior to the events).

TRAVELLING

Smoking Policy

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

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.

Tourism and Leisure

For further information about the city, please visit the following websites:
www.portoturismo.pt
www.portoenorte.pt
http://www.lonelyplanet.com/portugal/the-north/porto/things-to-do

SCIENTIFIC PROGRAM

Day 1 | Monday, October 25th	
09:30 - 09:45	**Registration**
09:45 - 10:00	**Welcome and opening ceremony** Rui L. Reis and Manuela E. Gomes (3B´s Research Group, University of Minho, Portugal)
	Session I **Chairs: Manuela E. Gomes & Rui L. Reis**
10:00 - 11:00	**Recreating the tendon niche *in vitro*** Dimitrios I. Zeugolis (University College Dublin, Dublin, Ireland)
11:00 - 11:30	**Coffee-Break | E-Poster Session**
	Session II **Chairs: Ana I. Gonçalves & Manuel Gomez-Florit**
11:30 - 12:30	**Stem cell therapy in tendinopathy – backing the right horse?** Roger K.W. Smith (The Royal Veterinary College, London, UK)
12:30 -14:30	**Lunch**
14:30 -15:30	**Gene and Transcript Therapies for Tendon Repair and Regeneration** Elizabeth Rosado Balmayor (Maastricht University, Netherlands)
	Short Oral Presentations
15:30 -15:40	**Umbilical cord mesenchymal stromal cell-derived small extracellular vesicles improve rotator cuff healing - a pilot ovine study** Andreas Traweger (Paracelsus Medical University, Austria)
15:40 - 15:50	**IL4-decorated SPIONs contribute to immunoregulatory actions on macrophages via IL4/STAT6** Ana F. Almeida (3B's Research Group, University of Minho, Portugal)

15:50 -16:00	**miRNA signature of injured entheses in an animal model** Carlos J. Peniche Silva (Maastricht University, Netherlands)
16:00 - 16:30	**Coffee-Break | E-Poster Session**
	Session III **Chairs: Elizabeth Balmayor & Roger Smith**
16:30 - 17:30	**Transitional tendinopathy: are we there yet?** Neal L. Millar (University of Glasgow, UK)
17:30 - 18:30	**Get-together Reception**

Day 2 | Tuesday, October 26th	
	Session I **Chairs: Manuela E. Gomes & Dimitrios I. Zeugolis**
09:30 - 10:30	**Enhancement of tendon healing - from biology to surgery** Paul W. Ackermann (Karolinska University Hospital, Sweden)
10:30 - 11:00	**Coffee-Break | E-Poster Session**
	Session II **Chairs: Christopher Evans & Manuel Gomez-Florit**
11:00 – 12:00	**Cell stressors in tendinopathies and implications for repair strategies** Jay Dudhia (The Royal Veterinary College, London, UK)
	Short Oral Presentations
12:00 -12:10	**Spider silk proteins with tenogenic peptides inspires a new class of bioengineered materials for tendon applications** Albina R. Franco (3B's Research Group, University of Minho, Portugal)

12:10 - 12:20	**Tendon-like electrospun PLGA scaffolds with optimized physical cues induced tenogenic differentiation and boosted immunomodulatory properties on amniotic epithelial stem cells** Mohammad El Khatib (University of Teramo, Italy)
12:20 -12:30	**Providing polymeric hydrogels with anisotropic properties through the incorporation of extremely-high magnetic power nanomaterials for tendon tissue engineering applications** Alberto Pardo (3B's Research Group, University of Minho, Portugal)
12:30 – 14:30	**Lunch**
	Session III **Chairs: Jay Dudhia & Ana I. Gonçalves**
14:30 - 15:30	**Magnetically-assisted approaches to address inflammatory events in tendon lesions** Márcia T. Rodrigues (3B's Research Group, University of Minho, Portugal)
15:30 - 16:30	**Regenerating tendons: lessons from development** Alice Huang (Columbia University, NY, USA)
16:30 – 17:00	**Coffee Break | E-Poster Session**
	Session IV **Chairs: Márcia T. Rodrigues & Neal L. Millar**
	Transversal Skills Training
17:00 – 17:30	**Ethics in animal experimentation: when and how to use animal models in pre-clinical studies** Magda J. Castelhano-Carlos (School of Medicine/ICVS, University of Minho, Portugal)
17:30 – 18:30	**Progress meeting Achilles (restricted meeting)**

Day 3 | Wednesday, October 27th	
	Session I **Chairs: Alexandre Barros & Rui M. A. Domingues**
	Transversal Skills Training
09:30 - 10:00	**Advanced therapies from the perspective of a Brazilian Company** Marcos Ribeiro (Sintegra Surgical Sciences, Brasil)
10:00 – 10:30	**Intelectual Property protection and Patent Strategy** Anabela Carvalho (PATENTREE, Porto, Portugal)
10:30 - 11:00	**Coffee-Break | E-Poster Session**
11:00 -12:00	**Getting published in Wiley's Materials Science Journals: Tips and Tricks for Surviving Peer Review** Valentina Lombardo (Wiley)
12:00 – 12:30	**Closing ceremony**
12:30 – 14:30	**Lunch**

KEYNOTE LECTURES & MASTER CLASSES

RECREATING THE TENDON NICHE *IN VITRO*

Dimitrios I. Zeugolis

Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), Charles Institute of Dermatology, Conway Institute of Biomolecular & Biomedical Research and School of Mechanical & Materials Engineering, University College Dublin (UCD), Dublin, Ireland

Traditional *ex vivo* culture conditions fail to imitate the native tissue niche leading to cellular senescence, phenotypic drift and loss of cellular phenotype, function and therapeutic potential. This talk will discuss the influence of biophysical (e.g. surface topography, substrate rigidity, mechanical stimulation, macromolecular crowding), biochemical (e.g. oxygen tension) and biological (e.g. growth factor supplementation) signals, alone or in combination, in controlling cell fate in the tendon context.

Dimitrios I Zeugolis Short CV

Dimitrios I. Zeugolis is the Director of the Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), Full Professor at the School of Mechanical & Materials Engineering, Principal Investigator at the Charles Institute of Dermatology and Senior Fellow at the Conway Institute of Biomolecular & Biomedical Research, all at University College Dublin (UCD), IE. He is Adjunct Professor at the National University of Ireland Galway (NUI Galway), IE and Co-Director of the Laboratory of Animal Science, Nutrition and Biotechnology, University of Ioannina, GR. He is Editorial Committee member of the Tissue Engineering and Regenerative Medicine International Society (TERMIS), Irish Ambassador of the European Orthopaedic Research Society (EORS) and Council Member of Matrix Biology Ireland (MBI). He is also Editor-in-Chief of Biomaterials and Biosystems (Elsevier), Senior Editorial Board member of BMC Biomedical Engineering (Springer Nature) and Associate Editor of Frontiers in

Bioengineering and Biotechnology. Dimitrios is also Pool of Experts Board of Biotechnology and Biological Sciences Research Council (BBSRC), UK and Review College Board of Research Foundation Flanders (FWO), BE. REMODEL research themes are: (A) Biomaterial- and cell- based therapeutic, reparative and regenerative products for healthcare. (B) *In vitro* pathophysiology models for drug discovery. (C) Cellular agriculture and aquaculture meat products for food security. (D) Tools and technologies that enable effective and efficient *ex vivo* cell propagation for the cell culture sector.

STEM CELL THERAPY IN TENDINOPATHY – BACKING THE RIGHT HORSE?

Roger K.W. Smith

The Royal Veterinary College, London, UK

There is growing interest in using mesenchymal stem/stromal cells (MSCs) for the treatment of soft tissue injuries. The overall aim of this therapy has been to encourage the regeneration of tissues post injury and, while there is little evidence true regeneration is induced by these cells, there is increasing evidence that they can still improve healing through a variety of mechanisms. The strongest evidence is that MSCs modulate inflammation within the tissue, key factors in the persistent and recurrent signs of tendinopathy in both equine and human tendinopathy [1, 2]. These immunomodulatory effects are most likely mediated via the release of paracrine mediators, such as extracellular vesicles.

Naturally-occurring equine superficial digital flexor tendon (SDFT) overstrain injuries in the horse usually have a contained lesion, thereby enabling simple intra-tendinous injection. The equine injury has many similarities to human Achilles tendinopathy although the correlates are matched more by function than by anatomy due to the differences in tendon loading between bipeds and quadrupeds. However, this makes the horse a useful model for human tendon disease. Mesenchymal stem cells have been in use clinically in the horse since the first reported use in 2003 [3] due to a more relaxed regulatory framework. There are multiple autologous cell options (riders in the race) used clinically, differing by the source tissues but also by their method of preparation – either 'minimally manipulated' through direct extraction of a cellular component through enzymatic treatment of the tissue (eg fat) versus *ex vivo* culture, usually of bone marrow. The latter provides a better defined and more homogeneous cell population that can be combined with bone marrow supernatant to provide an additional growth factor stimulus. Most recently, allogenic cell products have received

European marketing authorisation but are only currently licensed for the treatment of osteoarthritis and not for tendon or ligament lesions.

To test the hypothesis that MSCs will enhance tendon healing, a controlled experimental study of naturally-occurring SDFT injuries (n=12) has been performed [4]. MSC treatment appeared to 'normalise' many of the relevant tissue parameters so that they were closer to the contralateral, relatively normal, and untreated tendons than saline-injected controls, despite labelling experiments showing most cells being lost within 24 hours [5, 6]. A second adequately powered and independently analysed study evaluated the clinical outcome of naturally occurring SDFT injuries treated using this technique (n=113) which showed a significantly reduced re-injury rate [7]. This data has been used to support a human Phase IIa clinical trial for Achilles tendinopathy [8] using the same technique. The results of this study showed the technique to be safe and resulted in minimal clinically important difference of >12pts ($p<0.05$) improvement achieved in 8/10 patients.

In contrast to apparent beneficial results for the treatment of extra-thecal tendinopathy, the 'race' is very different for intrasynovial (intra-thecal) tendon pathology (eg rotator cuff injuries in humans; deep digital flexor tendon tears in horses) because of the challenges presented by the synovial environment [9] and the lack of a paratenon. Experimental administration of MSCs intra-synovially have failed to improve healing in an ovine (induced) critical defect tendon model [10]. Labelling of the implanted cells showed them to lodge within the synovium with no cells present in the tendon defect itself. In contrast, these lesions can be made to heal using acellular scaffolds [11], indicating that cellular therapies may not have universal application for all tendon pathologies.

References: [1] Dakin, S.G., et al., *Macrophage sub-populations and the lipoxin A4 receptor implicate active inflammation during equine tendon repair.* PLoS One, 2012. 7(2): p. e32333. [2] Dakin, S.G., et al., *Inflammation activation and resolution in human tendon disease.* Sci Transl Med, 2015. 7(311): p. 311ra173. [3] Smith, R.K., et al., *Isolation and implantation of autologous equine mesenchymal stem cells from bone marrow into the superficial digital flexor tendon as a potential novel treatment.* Equine Vet J, 2003. 35(1): p. 99-102. [4] Smith, R.K.W., et al., *Beneficial effects of autologous bone marrow-derived mesenchymal stem cells in naturally-occurring tendinopathy.* PLoS One, 2013. 8: p. e75697. [5] Becerra, P., et al., *Distribution of injected technetium(99m) -labeled mesenchymal stem cells in horses with naturally occurring*

tendinopathy. J Orthop Res, 2013. 31(7): p. 1096-102. [6] Sole, A., et al., *Distribution and persistence of technetium-99 hexamethyl propylene amine oxime-labelled bone marrow-derived mesenchymal stem cells in experimentally induced tendon lesions after intratendinous injection and regional perfusion of the equine distal limb.* Equine Vet J, 2013. 45(6): p. 726-31. [7] Godwin, E.E., et al., *Implantation of bone marrow-derived mesenchymal stem cells demonstrates improved outcome in horses with overstrain injury of the superficial digital flexor tendon.* Equine Vet J, 2012. 44(1): p. 25-32. [8] Goldberg, A.J., et al., *Autologous Stem Cells in Achilles Tendinopathy (ASCAT): protocol for a phase IIA, single-centre, proof-of-concept study.* BMJ Open, 2018. 8(5): p. e021600. [9] Garvican, E.R., et al., *Exposure of a tendon extracellular matrix to synovial fluid triggers endogenous and engrafted cell death: A mechanism for failed healing of intrathecal tendon injuries.* Connect Tissue Res, 2017. 58(5): p. 438-446. [10] Khan, M.R., et al., *Bone marrow mesenchymal stem cells do not enhance intra-synovial tendon healing despite engraftment and homing to niches within the synovium.* Stem Cell Res Ther, 2018. 9(1): p. 169. [11] Rashid, M., et al., *Histopathological and immunohistochemical evaluation of cellular response to a woven and electrospun polydioxanone (PDO) and polycaprolactone (PCL) patch for tendon repair.* Sci Rep, 2020. 10(1): p. 4754.

Roger K.W. Smith Short CV

Roger Smith is Professor of Equine Orthopaedics at the Royal Veterinary College, London, UK. He qualified as a veterinary surgeon from Cambridge University (UK) in 1987, having obtained a First for his undergraduate degree and a Cambridge Blue at swimming. After 2 years in practice, he returned to academia to undertake further clinical training as a Resident in Equine Studies at the Royal Veterinary College. Following his residency, he undertook a 3 year research project culminating in the award of a PhD for his studies on the extracellular matrix of equine tendon. He remained at the Royal Veterinary College, first as a Lecturer in Equine Surgery, then as Senior Lecturer in Equine Surgery before his appointment as Professor in Equine Orthopaedics in December 2003. He holds the Diploma of Equine Orthopaedics from the Royal College of Veterinary Surgeons and is a Royal College of Veterinary Surgeons Specialist in Equine Surgery. He is a Diplomate of the European Colleges of Veterinary Surgeons and Veterinary Sports Medicine and Rehabilitation, and is also a Large Animal Associate of the European College of Veterinary Diagnostic Imaging. In 2016, he was awarded the Fellowship of the Royal College of Veterinary Surgeons for meritorious contribution to knowledge and was elected to president of the European College of

Veterinary Surgeons in July 2017. He divides his time between running a specialist orthopaedic referral service within the Royal Veterinary College, where he is involved in lameness diagnostics, imaging and orthopaedic surgery, and continuing to direct research into equine tendon disease. His principal research interests are understanding the pathogenesis of tendon disease, diagnostics for tendon and ligament disease, and stem cell therapy for tendons in both horses and humans. He is married to a medical doctor and has two sons.

GENE AND TRANSCRIPT THERAPIES FOR TENDON REPAIR AND REGENERATION

Elizabeth Rosado Balmayor
Department IBE, MERLN, Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, Netherlands

Tendon injuries are common, with the highest incidence in young adults below the age of 30. Most tendon injuries are the result of gradual wear and tear occurring from overuse of the tissue or aging. They may lead to tendinopathy or tendon rupture, and are generally accompanied by significant disability, pain, healthcare cost, and lost productivity. Repair of diverse tendon injury types in experimental models has been significantly improved by the use of growth factors. Clinical use of growth factors normally requires their administration at very high doses that cause adverse side effects and raise costs while providing only incremental benefit. Furthermore, keeping the growth factors local is cumbersome in the tendon environment. Local gene delivery provides a promising, alternative approach to delivering proteins at therapeutic levels within a tendon lesion for an extended period of time. This can be pursued by delivering plasmid DNA using viral or non-viral vectors. Significant preclinical progress has been made, but the disadvantages of current genetic delivery strategies are the cost, safety concerns, and the regulatory complexity of clinical translation. Messenger RNA (mRNA) is a new class of drugs that can be used to express a therapeutic protein and, in contrast to DNA, is safer and inexpensive.

This presentation seeks to introduce the students to the gene and transcript therapy concepts, their advantages, and their limitations. Several examples of current research will be provided to illustrate the uses of protein-coding plasmid DNA and mRNA for tendon repair and regeneration. Future perspectives will be highlighted.

Elizabeth Rosado Balmayor Short CV

Elizabeth Rosado Balmayor has been Assistant Professor at the MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University since 2019. She was trained as a Chemist and earned a M.Sc. in Materials Science and Technology at the

University of Havana (Cuba). She received a Marie Curie scholarship in the area of Biomaterials and completed her Ph.D. in 2009 with Prof. Rui Reis at the 3B's research group in Portugal. Elizabeth obtained her assistant professorship in Experimental Trauma Surgery from TUM after concluding her Habilitation (Germany) in 2017. She is associate researcher with Prof. Chris Evans at the Mayo clinic (USA) and holds visiting professorships at the Peruvian University Cayetano Heredia and at the UNESCO Biomaterials' chair of the University of Havana. Her achievements have been recognized with a number of awards and grants, including the Hans-Liniger-Award by the German Society for Trauma Surgery, and projects from the German Federal Ministry for Economic Affairs and Energy, European H2020 funding scheme and the U.S. National Institutes of Health - NIH. She has obtained over 3.5 million euros from competitive grants for her research.

A powerful breakthrough in Elizabeth's research is the development of a chemically modified mRNA encoding BMP-2 as an alternative to traditional gene therapy for bone healing. She is a pioneer in the application of this novel technology to tissue regeneration and holds a patent on this discovery, that served as the basis for the creation of the start-up company Ethris GmbH of which she is one of the founders.

Elizabeth has published 62 publications in peer-reviewed scientific journals and authored 8 book chapters in books with international circulation. She is the author of 2 patents. In addition, she is associate editor of the European Journal of Medical Research and of Frontiers in Bioengineering and Biotechnology. She has been invited editor of special issues for the journals Advanced Drug Delivery Reviews, Tissue Engineering Part A, Frontiers in Bioengineering and Biotechnology, and International Journal of Molecular Sciences. Elizabeth has been a board member and young scientists' representative at the International Graduate School of Science and Engineering at TUM, and she is currently the women leadership chair and ex-officio board member of EORS. She is also part of the council board of TERMIS EU, and she has served as Auditor for the society from 2017 – 2019. She is also part of the ORS International Section of Fracture Repair Communications Committee.

TRANSITIONAL TENDINOPATHY: ARE WE THERE YET?

Neal L Millar

University of Glasgow, UK

This lecture will explore the recent inflammatory discoveries in tendon disease and discuss how this may lead to real patient benefit in clinical translational trials.

Neal L Millar Short CV

Neal L Millar PhD FRCSEd(Tr&Orth) is Professor of Orthopaedic Surgery and an Academic Consultant Orthopaedic Surgeon based at the University of Glasgow, specialising in shoulder surgery and tendon injuries having completed fellowships in Sydney and New York. His laboratory's research focuses on the immunopathogenesis and translational immunobiology of soft tissue musculoskeletal diseases including tendinopathy. He has been instrumental in leading/designing clinical trials of novel therapies in human tendon disease. He has completed a worldwide Phase II clinical trial of IL-17A blockade in shoulder tendinopathy patients following his laboratory discovery of a key role of IL-17 in tendon disease. Furthermore his discovery of a single microRNA-dependent regulatory pathway in early tissue healing highlights *a microRNA* replacement therapy as a promising therapeutic option for human tendon disease currently Phase I human development representing true 'translational' science. Additionally, he runs a specialist 'One stop' complex tendon clinic in the NHS focused on improving the treatment of tendinopathy.

ENHANCEMENT OF TENDON HEALING - FROM BIOLOGY TO SURGERY

Paul W. Ackermann

Dept of Molecular Medicine and Surgery, Karolinska Institutet and Dept of Orthopedic Surgery, Karolinska University Hospital, Sweden

Failed tendon healing and tendinopathy are disorders increasing in prevalence, which still pose as enigmas for both patients and therapists. Several novel as well as improved versions of old therapies rapidly become available for the use of enhancing tendon repair. Although novel tissue engineering and tissue regenerative techniques addressing tendon repair seem promising, these are not yet ready for routine clinical use. Such methods include molecular approaches by which PRP, PRF, fibrin glue, including stem cells, synthesize growth factors or other mediators needed for progression of failed healing.

Fundamental reasons to the big gap between successful laboratory therapies and failures in clinical trials may partly lie in the varying underlying human pathology. Human tendon pathology may be associated with disorders such as genetic variants of matrix proteins, metabolic disorders and neuronal dysregulation (neuropathy), which contribute to failed healing and the development of tendinopathy.

Essential factors to successful enhancement of tendon repair thus involve understanding and targeting of underlying pathology, surgical factors, modification of factors during the healing phases, as well as factors during post-surgery and rehabilitation phases. I will be addressing most of these factors in my talk during the "Third Achilles Workshop" in Porto.

Paul W. Ackermann Short CV

Education

2009 Qualification as Associate Professor, Karolinska Institutet
2002-2008 Postdoctoral work: Assistant Professor, "Neuronal Role in Musculoskeletal Healing", Karolinska Institutet and collaboration with University of Calgary
2001 Doctoral degree: Orthopedics/ Sportsmedicine, "Peptidergic Innervation of Periarticular Tissue" at Karolinska Institutet
1998 M.D: Medical school at Karolinska Institutet

Current Positions

2021 - Professor at Karolinska Institutet and Karolinska University Hospital
2012 - Consultant Orthopedic Surgeon, Orthopedic Traumatology Care, Karolinska University Hospital
2009 - Head of the Integrative Orthopedic Research Group, Karolinska Institutet
2008 - Specialist in Orthopedic Surgery, Orthopedic Traumatology Care

Previous Positions

2002-2008 Resident in Orthopedic Surgery, Karolinska University Hospital
2000-2002 Internship/research, Karolinska University Hospital
1998-2001 PhD-student, Karolinska Institutet

Research areas and expertise

My research aim is to understand and exploit neuro-vascular pathways in the musculo-skeletal system for restoring joint function, alleviating pain and minimizing complications.
Chronic tendon pain, tendinopathy, and defective tendon repair are enigmas that are still puzzling us as clinicians and researchers.
Dr. P.W. Ackermann is the Head of the Integrative Orthopedic Research Group, focusing his research on Neuronal Regulation of Pain and Musculo-skeletal Tissue Repair in collaboration with a global network of researchers. His mission in research is to understand and exploit neuro-vascular pathways in the musculo-skeletal system for restoring joint function, alleviating pain and minimizing complications. Dr. P.W. Ackermann is a specialist in orthopedic surgery with focus on trauma. His mission is to develop and translate basic research findings into clinical practice.

CELL STRESSORS IN TENDINOPATHIES AND IMPLICATIONS FOR REPAIR STRATEGIES

Jay Dudhia

Department of Clinical Science and Services, Royal Veterinary College, University of London, UK

Tendon injuries in humans are a major healthcare concern but they also occur spontaneously in other species including horses. The superficial digital flexor tendon (SDFT) of the horse is a highly relevant model for studying tendinopathies because it is a functional homologue of the Achilles tendon and there are similarities in the aetiopathogenesis of injuries, associated risk factors and exacerbated by corticosteroid treatment. Corticosteroids are stressors that induce the senescent state with the production of pro-inflammatory cytokines (the Senescence Associated Secretory Phenotype or SASP) including TNF-α and IL-1β. SASP acts to clear senescent cells by the immune cells but is overlooked in tendon repair strategies. We have investigated hyperthermia in the SDFT as a stressor for apoptosis and cellular senescence and have further developed an in vitro engineered 3D tendon model for studying molecular mechanisms in tendinopathies and developing cell-based therapies. Repetitive and rapid cyclical loading during exercise is known to elevate temperature by 6 – 8 $^{\circ}$C in the core of the SDFT. In tendon explants treated in culture at 45 $^{\circ}$C for 10 min, to mimic in vivo conditions, both protective cellular mechanisms and apoptotic markers are increased at 2 h and sustained for up to 8 h. The increased expression pattern is confined within a linear row of interconnected cells along a fascicle but not in adjacent lateral rows. Gap junction expression is also elevated suggesting facilitation of crosstalk of protective and apoptotic signals along a row of cells but not laterally in adjacent parallel rows. Although staining for senescent-associated β-gal (SA-β-gal) was inconclusive, SA-β-gal was induced in tenocytes exposed to hyperthermia or dexamethasone. The effect of dexamethasone was reversed by resveratrol, a

polyphenol known to release senescent cells from cell cycle arrest suggesting that it may be possible to rescue senescent tenocytes to reduce their loss due to SASP or ageing. To further investigate molecular mechanisms, we have developed a 3D culture system that more closely mimics the in vivo environment. Tenocytes growing in collagen constructs contract the collagen, which is a measure of the cells ability to organise the extracellular matrix but is impaired by TNF-α and IL-1β. These cytokines also stimulate the nuclear translocation of NF-κB in tenocytes. We are currently investigating the complex interplay between NF-κB activity, which includes apoptosis and cell cycle control, its regulator SIRT1 and resveratrol as a potential intervention in repair strategies.

Jay Dudhia Short CV

Dr Jay Dudhia is Senior Lecturer in Stem Cell Biology at the Royal Veterinary College, University London. He has a PhD is Molecular Biology from University of London, and his early career interests at the Kennedy Institute of Rheumatology were in human osteoarthritis investigating age-related changes in articular cartilage and molecular markers for early disease. The work has led to a proof-of-concept study in patients with osteoarthritis for a novel arthrospectroscopy-based approach to image cartilage. In the past 15 years at the Royal Veterinary College his research focus has been on the biology and clinical applications of mesenchymal stromal cells for the treatment of tendinopathies in the horse and for joint diseases in the dog for routine use in the veterinary clinic. More recently he has been investigating cardiac derived cells and iPSCs in the dog and cat for cardiomyopathies. He heads a veterinary Stem Cell laboratory for the manufacture of clinical grade cell-based therapies within the newly established Vaccinology and Regenerative Medicine Centre at the Royal Veterinary College.

MAGNETICALLY-ASSISTED APPROACHES TO ADDRESS INFLAMMATORY EVENTS IN TENDON LESIONS

Márcia T. Rodrigues

3B's Research Group, University of Minho, Portugal

Tendon afflictions constitute a significant share of musculoskeletal diseases and a primary cause of incapacity worldwide. Unresolved/chronic inflammatory states have been associated with tendinopathic conditions and to hampered healing, contributing to undesirable immune stimulation and detrimental effects to the tissues. Successful treatment of tendon lesions and improved regenerative solutions may rely on a more comprehensive understanding and guidance of inflammatory events.

Magnetically-assisted nanotechnologies embrace sophisticated tools as magnetic iron oxide nanoparticles with the potential to modulate tissue and cell responses combining contactless control, high precision, and tissue penetration for tracking, controlled release, and real-time monitoring. We have been exploring magnetically-assisted technologies to guide cell behavior and in the regulation of inflammatory mechanisms, anticipating the applicability of magnetically-assisted therapies and the successful integration of tissue-engineered substitutes for tendon regeneration.

In this talk, we will review tendon models of different complexity to study the cellular and molecular responses to inflammatory triggers and to predict tendon niche responses resourcing to magnetic stimulation, alone or combined with magnetically responsive nanomaterials to modulate pathophysiological responses. These models include single and co-cultures of tendon-derived cells primed with pro-inflammatory mediators, magnetic cell sheet constructions that provide a more tissue-like environment with intercellular signaling and structural complexity, and magnetic responsive polymeric membranes to support tendon-derived cell responses.

Considering the impact of macrophages in the inflammatory process, and in promoting tissue repair, we will also present magnetically responsive nanotools to tackle inflammation and engage macrophages into pro-regenerative phenotypes, anticipating more efficient treatments for tendinopathy conditions.

Márcia T. Rodrigues Short CV

Márcia T. Rodrigues holds a degree in Applied Biology, a Ph.D. in Tissue Engineering, Regenerative Medicine, and Stem Cells, and is currently an Assistant Researcher at the 3B's Research Group in the University of Minho in Portugal.

MT Rodrigues has been investigating strategies to study inflammatory signals associated with tendon disorders resourcing to magnetically-assisted technologies. She is particularly interested in platforms for remotely controlled cell guidance and modulation of inflammatory events, for magnetically controlled release of bioactive payloads, and in cellular reprogramming tools for guided therapies. The studies on immunomodulatory actions also hold the potential to improve the integration and lifespan of tendon substitutes and artificial devices at the injury site.

She has integrated the team of several national and European projects in both research and educational activities, and has been invited to lecture seminars in symposia and in post-graduation courses. Presently, she is editor of 1 book in tendon regeneration (Elsevier, 2015), and author of 13 book chapters, 48 full papers in international peer-reviewed journals, and over 85 communications.

REGENERATING TENDONS: LESSONS FROM DEVELOPMENT

Alice Huang

Department of Orthopedic Surgery, Columbia University, USA

Adult tendon heals via fibrosis, and this failure to re-establish native tendon structure is likely the leading cause of injury recurrence. To date, most models of tendon injury are models of poor, fibrotic healing, which limits the ability to identify regenerative mechanisms. In recent work, we showed that neonatal mice are able to regenerate functional tendons, however regenerative capacity is lost in adult stages with tendon maturation. Using these models, we are testing mechanisms that distinguish neonatal regeneration from adult scar formation. In particular, we have identified differences in intrinsic tenocyte capacity, the immune environment, and signaling pathways that may drive regeneration. Using developmentally-directed differentiation of pluripotent stem cells, we are also establishing protocols for generating 'regenerative' tenocytes in vitro for adult tendon repair.

Alice Huang Short CV

Dr. Alice Huang is currently an Associate Professor in the Department of Orthopedic Surgery at Columbia University. Dr. Huang graduated from Barnard College and the School of Engineering and Applied Science at Columbia University with a B.A. in Asian/Middle Eastern Studies and a B.S. in Biomedical Engineering. She then completed her Ph.D in Bioengineering at the University of Pennsylvania. Following the completion of her graduate studies, Dr. Huang conducted postdoctoral research in Developmental Biology at Shriners Hospital for Children, where she investigated mechanisms of musculoskeletal development and integration during embryogenesis. In 2014, Dr. Huang joined the faculty at Mount Sinai as an Assistant Professor in the

Department of Orthopaedics, with a secondary appointment in Developmental and Regenerative Biology. Dr. Huang moved her lab to Columbia University in 2021.

Dr. Huang's group combines tools and approaches from developmental biology and tissue engineering to study regenerative and non-regenerative healing of musculoskeletal tissues. Dr. Huang's team is especially interested in understanding the cell and molecular mechanisms that regulate development, regeneration, and engineering of fibrous connective tissues, such as tendons/ligaments/annulus fibrosis, which have been relatively understudied. Dr. Huang has trained undergraduates, PhD and MD/PhD graduate students, postdoctoral fellows, and Orthopaedic residents. Her research has been funded by the National Institutes of Health (NIH), New York State Stem Cells (NYSTEM), and the Orthopaedic Research and Education Foundation (OREF). She has received numerous awards for her work, including the Mount Sinai Faculty Award and the Kappa Delta Young Investigator Award from the American Academy of Orthopaedic Surgeons.

TRANSVERSAL SKILLS TRAINING

ETHICS IN ANIMAL EXPERIMENTATION: WHEN AND HOW TO USE ANIMAL MODELS IN PRE-CLINICAL STUDIES

Magda J. Castelhano-Carlos

School of Medicine/ICVS, University of Minho, Portugal

The lecture will focus on the use of animal models and how ethically justifiable it can be for pre-clinical studies, as well as in important aspects of nowadays regulations and laboratory animal science developments and a culture of care contributing for science quality.

M. J. Castelhano-Carlos Short CV

Coordinator of the Animal Facilities Unit at the School of Medicine and Life and Health Sciences Research Institute (EM/ICVS) of the University of Minho (UM), M.J. Castelhano-Carlos graduated in Biochemist (Porto University, Portugal), did a Master in Laboratory Animal Science (Veterinary Faculty, Utrecht University, Netherlands), and a PhD in Health Sciences (at the EM/ICVS, UM). Her research interests cover animal welfare and behaviour, laboratory animal sciences (LAS) and neuroscience, as well as automation and management applied to research animal facilities. M.J. Castelhano-Carlos is also responsible for designing and co-organizing LAS post-graduation training courses at the EM/ICVS-UM since 2004, teaching several subjects in those courses, as well as in other courses in Minho University and in other national and international courses, including lectures on ethics in animals based research. She is an active member of the Portuguese Society for Laboratory Animal Science – SPCAL (elected president since April 2021); member of the FELASA Education and Training Accreditation Board and member of the ETPLAS Stakeholders Board.
ORCID ID: https://orcid.org/0000-0002-5409-5987

ADVANCED THERAPIES FROM THE PERSPECTIVE OF A BRAZILIAN COMPANY

Marcos Antonio Ribeiro

Sintegra Surgical Sciences, Brasil

This lecture will explore the following topics of discussion:

- Our Company
- The Brazilian Health Market
- The regulatory process for Advanced Therapies
- Needs and Opportunities

INTELECTUAL PROPERTY PROTECTION AND PATENT STRATEGY

Anabela Carvalho

Qualified European Patent Attorney
Portuguese Official Industrial Property Agent
European Trademark and Design Attorney

Intelectual Property protection is a key aspect for inventors or investors, so they can protect their inventions and reap the full benefits of their creations and investment. Therefore, it is of utmost importance to think carefully on the IP strategy and be aware of the different alternatives available nowadays to do so. The aim of this presentation is to give an overview of intellectual property protection strategies and "do's" and "don'ts", with focus on patents on the field of Tissue Engineering and Regenerative Medicine. Some examples of marketed outcomes will be also analyzed, which may be helpful for those who intend to develop, and protect, innovative tissue-engineered products.

Anabela Carvalho Short CV

Anabela Carvalho is a qualified European patent attorney, a Portuguese patent and trademark attorney, a Portuguese industrial property agent and a European trademark and design attorney. She is also a patent arbitrator. In 2013 Ms Carvalho was one of the first few professionals in Portugal to pass the European qualifying exam.
Ms Carvalho holds a bioengineering degree from the University of Minho, Portugal. She is a partner at PATENTREE.
Ms Carvalho has interned with the EPO Examining Division. She is a tutor for the Lisbon branch of the Centre for International Intellectual Property Studies and a tutor for the Institute of Professional Representatives of the EPO.

Ms Carvalho has helped clients extensively in the development of strategies and solutions for patent and IP management (advanced and defensive patent filings). She has comprehensive patent prosecution experience in European, Patent Cooperation Treaty and Portuguese patent applications, including providing opinions and analyses on patentability, freedom-to-operate and infringement matters. She also has experience as a judicial patent expert appointed both by the courts and by third parties.

GETTING PUBLISHED IN WILEY'S MATERIALS SCIENCE JOURNALS: TIPS AND TRICKS FOR SURVIVING PEER REVIEW

Valentina Lombardo

Wiley Online Library

Great ideas, successful experiments, good data analysis: everything is ready and you want to share your findings with the scientific community. You are all set and writing your scientific article should not be a big deal, right? However, sometime it is not the smooth process you had in mind, and you end up receiving negative feedback from editors and reviewers. Why does this happen? Publishing in scientific journals is not always easy. The editorial assessment and the peer review process are the crucial steps in which your research is questioned (or in other words, this is the part of the game that can give you nightmares). Luckily, there is light at the end of the tunnel and getting into the editor's mind can guide you through the preparation and the publication of your article. This presentation will help you to better understand the peer review process and provide you with some tips and tricks to get published more easily: what editors look for in your paper and why, what you should highlight and what you should (never!) do in order to write a good scientific article.

Valentina Lombardo Short CV

Valentina Lombardo obtained her B. Sc. Industrial Chemistry and a M.Sc. in Chemistry of Materials. She then completed her Ph.D. in Materials Science and Nanotechnology at the University of Catania in close collaboration with the Italian National Research Council - Institute for the Microelectronics and Microsystems. Her research activity was

mostly focused on conductive polymers for energy conversion and generation, and block copolymers self-assembly for nanolithography.

She joined Wiley in 2019 as peer review editor for materials science and physic journals, such as “Advanced Materials”, “Small”, “Advanced Materials Technologies” and “Advanced Engineering Materials”.

SHORT ORAL PRESENTATIONS

UMBILICAL CORD MESENCHYMAL STROMAL CELL-DERIVED SMALL EXTRACELLULAR VESICLES IMPROVE ROTATOR CUFF HEALING - A PILOT OVINE STUDY

F. Jenner[1,3], A. Wagner[2,3], I. Ribitsch[1,3], E. Ludewig[4], R. Trujanovic[5], E. Rohde[6,7], B. von Rechenberg[8,9], M. Gimona[7,10], A. Traweger[2,3]

[1]VETERM, Equine Surgery Unit, Department for Companion Animals and Horses, University of Veterinary Medicine Vienna; [2]Institute of Tendon and Bone Regeneration, Paracelsus Medical University; [3]Austrian Cluster for Tissue Regeneration; [4]Diagnostic Imaging Unit, Department for Companion Animals and Horses, University of Veterinary Medicine Vienna; [5]Clinical Unit of Anaesthesiology and perioperative Intensive Care, Department for Companion Animals and Horses, University of Veterinary Medicine Vienna; [6]Department of Transfusion Medicine, Salzburger Landeskliniken GesmbH;[7]GMP Unit, Spinal Cord Injury and Tissue Regeneration Centre Salzburg, Paracelsus Medical University; [8]Musculoskeletal Research Unit (MSRU), Vetsuisse Faculty, University of Zurich; [9]Center for Applied Biotechnology and Molecular Medicine (CABMM), University of Zurich; [10]Research Program "NanovesicularTherapies", Paracelsus Medical University.

Chronic and acute rotator cuff (RC) pathologies result in considerable disability, poor quality of life, and expensive utilization of health care resources. Injuries and degenerative changes of the RC are the most prominent cause of shoulder disability, and prevalence dramatically increases with age. The inflammatory milieu promotes infiltration and activation of immune cells, as well as the appearance of microvasculature and nerve fibers, resulting in substantial pain and complicating the effective management of rotator cuff tears (RCTs). Despite significant advancements in surgical techniques, failure rates remain high and novel approaches to adequately overcome the natural biological limits of tendon and enthesis regeneration at the rotator cuff are required to improve clinical outcomes. Small extracellular vesicles (sEVs) derived from the secretome of human multipotent mesenchymal stromal cells (MSCs) have been demonstrated to exert anti-inflammatory and anti-fibrotic activities. In this pilot study, we evaluated the efficacy of clinical-grade human umbilical cord MSC-derived small EVs (hUC-MSC-sEVs) loaded onto a type I collagen scaffold in an ovine infraspinatus tendon defect model to improve RC healing. Six weeks postoperatively, the regeneration of the tendon-to-bone insertion was evaluated by magnetic resonance imaging (MR) and hard tissue histology. The local application of hUC-MSC-sEVs attenuated the formation of osteophytes at the injured tendon-to-bone interface and promoted neotendon formation. Furthermore, MRI suggested less inflammation at the

defect area after application of hUC-MSC-sEVs. Together, hUC-MSC-sEVs promoted tendon-to-bone healing in an ovine rotator cuff injury model.

IL4-DECORATED SPIONS CONTRIBUTE TO IMMUNOREGULATORY ACTIONS ON MACROPHAGES VIA IL4/STAT6

A. F. Almeida[1,2], M. S. Miranda[1,2], A. Vinhas[1,2], A. I. Gonçalves[1,2], M. T. Rodrigues [1,2], M. E. Gomes[1,2]

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Inflammation is a vital part of the immune system's response to injury however, uncontrolled activation of immune cells results in persistent inflammation signals with associated constraints to tissue regeneration. Macrophages (Mφ) represent a key population in the modulation of inflammation and promotion of tissue repair by shifting pro-inflammatory (M1φ) and anti-inflammatory/healing (M2φ) phenotypes depending on the nature of environmental signals [1]. Therefore, the delicate balance between macrophage phenotypes holds the successful transition from persistent inflammation to its regulation and resolution, being critical for effective tissue regeneration. Interleukin 4 (IL4) is a well-known key regulator of Mφ profiles, that boosts an anti-inflammatory and pro-healing phenotype [2]. Despite the potential of IL4 to favor a pro-regenerative response, there is a quite limited success in IL4 delivery approaches. Thus, commercially available superparamagnetic iron oxide nanoparticles (SP) were used to bound IL4 via carbodiimide chemistry (SPIL4) enabling contactless control, monitoring and local retention [3]. The system was physical chemical characterized for dimension, shape, and surface charges as well as for IL4 binding efficiency. THP1-derived macrophages were also used to investigate viability and the profile of immune-modulatory molecules in the presence of SPIL4. Two time-points (1h or 24h) were investigated as well as two SP concentrations (30 or 100 μg/ml) to assess the impact of IL4 to drive macrophages phenotype. These outcomes were compared against exogenous IL4 (Exo IL4)-stimulated THP1. A magnetic field (MF) was provided by a magnefect device (nanoTherics Ltd, UK) (magnetic induction of 350 mT/ well). SPIL4 were shown to contribute for immune strategies participating in M2φ polarization via IL4/pSTAT6 pathway. Specifically, SPIL4-treated macrophages showed increased expression of IL10 and ARG1 genes, and of CCL2 and IL1Ra proteins, typically associated to M2φ, in comparison to soluble IL4 (Exo IL4), highlighting the effectiveness and impact of SPIL4 driving M2 signals. MF-assisted magnetic nanoparticle-protein systems offer new opportunities to use sophisticated nanotools to

cope with immune cells and interact on decisive cell signaling pathways with an active role in controlling inflammation mediators.

Acknowledgements: ERC CoG MagTendon (No. 772817), H2020 Twinning project Achilles (No. 810850), FCT under the Scientific Employment Stimulus - 2020.01157. CEECIND, and project Norte-01-0145-FEDER-02219015 supported by Norte Portugal Regional Operational Programme (NORTE 2020). Doctoral Grant SFRD/BD/144816/2019.

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MIRNA SIGNATURE OF INJURED ENTHESES IN AN ANIMAL MODEL

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Tendon attaches to bone through a highly specialized tissue called enthesis. Upon injury, the structural gradient of collagen alignment and mineralization of the enthesis is often not regenerated. As a result, scar tissue is formed that lacks the mechanical properties of the native tissue. Tissue engineers have approached this challenge from many angles. Our take on this challenge is based on the use of miRNAs as therapeutic tools to regulate gene expression. The use of miRNAs as epigenetic regulators has emerged as a promising strategy of molecular therapy, with numerous examples of miRNAs identified to influence tendon healing, cartilage formation/degradation, and even bone mineralization. With our study, we aimed to identify those miRNAs with therapeutic potential for enthesis regeneration by analyzing the miRNA expression profiles in healthy and injured tissue samples from a rat enthesis model. For this, we created a longitudinal defect in the patellar tendon enthesis of a rat and collected explants at 24 hours and 10 days after the injury. We investigated the expression of over 80 miRNAs at the two-selected time points through a pathway-focused miScript PCR array (Fibrosis). From the screened miRNAs, those found to be de-regulated over 2-fold in the injured samples compared to the native tissue were filtered by means of the Ingenuity Pathway Analysis software. This allowed us to narrow down our selection of miRNAs and predicted and/or validated mRNA targets with therapeutic potential for enthesis healing. Based on this, we investigated the expression of the mRNA targets and we found a correlation between the expression of the mir-16-5p, mir-17-5p, mir-124-3p, mir-133a-5p, mir-148a, miR-155-5p and mir-182, and their respective mRNA targets Smad3, Runx2, Mohawk, COL1a1, and COL2a1. Additionally, we investigated the protein expressions for structural collagens in the native and injured samples. As we expected from the miRNAs and mRNAs expression patterns, we found that the protein expression of collagen 1 and collagen 2 decreased significantly after 24 hours from the injury and increased back to healthy-like levels after 10 days. On the contrary, the expression of collagen 3 and collagen 10 was higher in the injured sample after 24h than after 10 days from the injury. We believe that our data bring important insights into the healing mechanism of injured entheses. Additionally, the selection of seven

miRNAs as potential candidates to address enthesis healing serves as stepping-stone to further research aiming at the development of novel miRNA-based therapy for enthesis regeneration.

ACHILLES
ADVANCING TENDON REGENERATIVE THERAPIES

SPIDER SILK PROTEINS WITH TENOGENIC PEPTIDES INSPIRES A NEW CLASS OF BIOENGINEERED MATERIALS FOR TENDON APPLICATIONS

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Tendon injuries constitute one of the most common musculoskeletal problems, where annually over 30 million tendon-related procedures take place [1]. Owning to the limited healing ability of these tissues and poor surgical repair treatment, they represent a major economic healthcare burden. Bioengineered proteins offer the opportunity to design novel multifunctional materials with tunable mechanical properties and biological features [2,3]. Spider silk, one of the most fascinating materials in nature, inspires a new class of functional synthetic polymers [4]. The aim of this study is to investigate the potential of bioengineer spider silk proteins with tenogenic peptides for tendon healing and repair. To achieve this purpose, domains derived from the dragline silk sequence of the spider *Nephila clavipes* (6mer) were fused with transforming growth factor β3 (TGFβ3) through step-by-step cloning. To test the bioactivity of produced bioengineered proteins, multifunctional films were prepared by combining different concentrations of bioengineered spider silk proteins (2% and 10%) with silk fibroin (8%; SF) extracted from *Bombyx mori* cocoons. The biological potential of the biomaterials was assessed using human adipose-derived stem cells (hASCs). The presence of 6mer-TGFβ3 shows higher transcript levels of tendon-related (*TNMD, SCX, Col-III, Col-I*) and chondrogenic-related (*Sox 9*, *ACAN*) genes. Also, the concentration of 6mer-TGFβ3 seems to affect the commitment of hASCs into the two different cells lineage. Regarding the films with 6mer or only SF, it was not observed the same behavior. Overall, we can conclude that the presence of bioengineered spider silk protein 6mer-TGFβ3 supports the expression of tenogenic and chondrogenic cues, evidencing cellular commitment towards tenogenic and chondrogenic lineages. Our findings provide new insights into the use of bioengineered spider silk with tenogenic peptides as building blocks for developing multifunctional living constructs aimed at tendon repair applications.

Acknowledgments: This work was supported by the Portuguese Foundation for Science and Technology (FCT) under the project PTDC/BII-BIO/28870/2017; A.R. Franco thanks grant SFRH/BPD/100760/2014.

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TENDON-LIKE ELECTROSPUN PLGA SCAFFOLDS WITH OPTIMIZED PHYSICAL CUES INDUCED TENOGENIC DIFFERENTIATION AND BOOSTED IMMUNOMODULATORY PROPERTIES ON AMNIOTIC EPITHELIAL STEM CELLS

M. El Khatib[1], V. Russo[1], G. Prencipe[1], A. Mauro[1], M.R. Citeroni[1], R. Wyrwa[2], P. Berardinelli[1], M. Schnabelrauch[2], B. Barboni[1]

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The advanced strategies in the field of Tissue Engineering might render possible overcoming the unsatisfactory results of conventional treatments to deal with tendinopathies. In this context, the design of tendon biomimetic electrospun scaffolds engineered with Amniotic Epithelial Stem Cells (AECs), which have shown a high teno-regenerative and immunomodulatory potential in tendon-defect models, can represent a promising solution for tendon regeneration. Poly(lactide-co-glycolic) acid (PLGA) scaffolds were fabricated using the electrospinning technique to mimic the native tendon biomechanics and extracellular matrix by optimizing: fiber alignment and diameter size (1.27 and 2.5 µm), and surface chemistry using the Cold Atmospheric Plasma (CAP) Technique. Moreover, the teno-inductive and immunomodulatory effects of these parameters on AECs have been also assessed. The fabricated PLGA scaffolds with highly aligned fibers and small diameter size (1.27 µm) induced a stepwise tenogenic differentiation on AECs with an early epithelial-mesenchymal transition (EMT), followed by their tenogenic differentiation. Indeed, SCX, an early tendon marker, was significantly more efficiently translated into the downstream effector TNMD, a mature tendon marker. Moreover, 1.27 µm fiber diameter induced on AECs a higher expression of anti-inflammatory interleukin mRNAs (*IL-4* and *IL-10*). The CAP treated PLGA scaffolds showed an improved cell adhesion and infiltration without altering their topological structure and teno-inductive properties. In fact, AECs engineered with CAP treated fibers, expressed in their cytoplasm TNMD. Moreover, CAP treatment did not alter the mechanical properties of PLGA scaffolds. The developed electrospun PLGA scaffolds with the optimized features represent an ideal tendon-like construct that could be applied in in-vivo models to evaluate their biosafety and teno-regenerative potential.

PROVIDING POLYMERIC HYDROGELS WITH ANISOTROPIC PROPERTIES THROUGH THE INCORPORATION OF EXTREMELY-HIGH MAGNETIC POWER NANOMATERIALS FOR TENDON TISSUE ENGINEERING APPLICATIONS

A. Pardo[1,2,3], *S. M. Bakht*[1,2], *S. P. B. Teixeira*[1,2], *M. Gomez-Florit*[1,2], *R. M. A. Domingues*[1,2], *M. E. Gomes*[1,2]

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Native tendon tissues are characterized by highly-anisotropic physical properties that are responsible for its biomechanical performance and biological organization. Hydrogels have been widely proposed as scaffolding materials, but its typical structure consisting on randomly-oriented polymeric networks results in isotropic properties different from those observed in biological systems. Remotely-addressable magnetic materials have the ability to provide hydrogels with anisotropic performance, resembling the characteristics of native extracellular matrices. Moreover, the intrinsic properties of magnetic nanoparticles (MNPs) enable their use as magnetomechanical actuators to control cellular/tissue behavior through the application of external magnetic fields. In this work, superparamagnetic iron oxide-based MNPs with magnetite-type structure and different magnetic behavior were obtained through thermal decomposition reactions. The particles displayed higher magnetization values were selected for their incorporation within electrospun polycaprolactone fibers. These hybrid fibers were then microcut and incorporated within gelatin solutions, applying an external magnetic field during the gelation process to force their uniaxial alignment. The fibers concentrations in the hydrogels were optimized in order to generate interdistances between them that allow cells proliferation and induce their elongated growth. In these preliminary studies, we focused on the design of MNPs with extremely-high magnetic power, thus allowing the use of low MNPs concentrations and weak magnetic field strengths/application times to fabricate the magnetically-responsive anisotropic constructs. In this way, in view of the potential transfer of the experiments to clinical practice, the toxic/safety risks associated with the use of high MNPs amounts and/or intense magnetic irradiations will be considerably reduced. Currently, magnetically-assisted 3D printing strategy is being explored to design anisotropic cell-laden constructs with controlled shape by applying a magnetic field during the printing process. The engineered nanocomposites replicate the uniaxial-anisotropic

extracellular matrix of native tendon tissues, being expected its effective performance as magnetomechanical remote actuators to control human adipose derived stem cells growth, migration and tenogenic differentiation. The design of hybrid fibers modified with weak-ferrimagnetic MNPs will also be explored in order to increase the effectiveness of cells remote stimulation.

Acknowledgements: Authors thank EC Twinning project - Achilles (810850), ERC CoG MagTendon (772817) and Fundacao para a Ciencia e Tecnologia (FCT) for Project SmarTendon-PTDC/NANMAT/ 30595/2017 and Project MagTT-PTDC/CTM-CTM/29930/2017. We also thank Xunta de Galicia for postdoctoral fellowship ED481B2019/025.

E-POSTER ABSTRACTS LIST

No. E-Poster	Presenting Author	Title
P01	S. Gimondi	Microfluidic-driven synthesis of size-controlled nanoparticles for anti-inflammatory purposes
P02	S. P. B. Teixeira	Directing stem cell commitment in 3d bioinspired hydrogels by growth factor sequestration using molecularly imprinted nanoparticles
P03	I. F. Cengiz	Silk-based 3D-printed scaffolds to treat meniscus defects
P04	R. R. Costa	Microfluidic-controlled interfacial complexation of collagen and glycosaminoglycans
P05	E. P. Oliveira	Dendrimers conjugated with manganese for ischemic stroke imaging
P06	R. Fontelo	Hemocompatibility of positively and negatively charged nanopatterned coatings
P07	S. M. Bakht	3D tendon-on-chip model to interrogate the multicellular crosstalk in healthy and diseased tendon
P08	R. F. Monteiro	Direct bioprinting of 3D human tendon models embedded on a biomimetic fibrillar matrix platform
P09	M. G. Fernandes	Ex-vivo static culture system to understand the mechanics of cutaneous scarring
P10	M. D. Malta	Signatures of dermal extracellular matrix of dystrophic epidermolysis bullosa patients
P11	A. L. Graça	Platelet-derived extracellular vesicles show therapeutic effects on a 3d tendon disease model
P12	L. Martins	Exploring extracellular matrix features of pemphigus vulgaris diseased skin cells
P13	M. C. Fonseca	Polyelectrolyte complexation of catechol containing polysaccharides for the development of bioadhesive membranes

P14	C. Gonçalves	Ionic liquid-assisted synthesis of porous saib/silk fibroin scaffolds for biomedical applications
P15	I. C. Silva	Synthesis and characterization of new quaternary bioactive glass nanoparticles for orthopedic applications
P16	V. I. B. Castro	Supramolecular hydrogels induce differentiation of stem cell into neural lineages
P17	S. Amaral	End-on conjugation of glycosaminoglycans to linear polymers for the preparation of ecm-like hydrogels
P18	S. Correia	Optimization of kefiran exopolysaccharide extraction for tissue engineering and regenerative medicine applications
P19	H. Radhouani	Methacrylated kefiran hydrogel for tissue engineering and regenerative medicine applications
P20	F. C. M. Lobo	Lignocellulosic compounds with antibacterial properties targeting environmental and biomedical surfaces
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E-POSTER ABSTRACTS

P01
MICROFLUIDIC-DRIVEN SYNTHESIS OF SIZE-CONTROLLED NANOPARTICLES FOR ANTI-INFLAMMATORY PURPOSES

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Within the nanotechnology field, nanoparticles (NPs) stand out due to their peculiar physicochemical properties. Therefore, the selection of the appropriate method to produce nanomaterials with the required properties is of paramount importance. Microfluidics can be exploited to achieve highly controlled NPs' synthesis. In this work, we employed a micromixer chip to generate NPs of poly(lactide-co-glycolide)-block-poly(ethylene glycol) (PLGA-PEG) with defined sizes. First, we investigated the role of flow rate and polymer concentration in the resulting NPs features. Then, three NPs sizes were selected for further studies, namely 30, 50, and 70 nm. NPs were fully characterized in terms of size, polydispersity index, surface charge, morphology, stability over time and loading capabilities of diclofenac, a non-steroidal anti-inflammatory drug (NSAID). Then, we evaluated the relation between NPs size and drug efficacy on the suppression of inflammatory pathways on lipopolysaccharide (LPS)-stimulated macrophages. This study shows that the size of polymeric NPs can have a significant impact on immune cells' inflammatory response and viability, suggesting that the precise control of NPs features can tailor the delivery of active agents and enhance their overall biological efficacy. The same platform was also used to synthetize polysaccharides-based NPs. Moreover, its efficacy was compared to the conventional dropwise method. Naturally active biomolecules, namely chitosan and high molecular weight hyaluronic acid, were selected due to their anti-inflammatory activity. NPs generated through the microfluidic technology were ≈2 times smaller and able to decrease more efficiently the levels of the main markers of inflammation (IL-1 α, PGE_2, IL-6, IL-8, MCAF, and TNF-α) on stimulated fibroblasts and macrophages than the NPs generated by the conventional method. Overall, our results demonstrate that the micromixer device can be used to enhance the synthesis of nanocarriers based on

bioactive macromolecules in order to exploit their intrinsic properties to fight inflammation and reduce side effects related to the common drugs.

Acknowledgements: This work was supported by the financial support of the FCT for the SG fellowship (PD/BD/143140/2019) and PATH (PD/00169/2013).

P02
DIRECTING STEM CELL COMMITMENT IN 3D BIOINSPIRED HYDROGELS BY GROWTH FACTOR SEQUESTRATION USING MOLECULARLY IMPRINTED NANOPARTICLES

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Growth factors (GFs) are one of the key components of tissue engineering, but their exogenous administration has proven costly and ineffective in therapeutic settings. Extracellular matrix (ECM)-inspired biomaterial approaches have sought to sequester these molecules, regulating their activity and presentation to cell receptors. Our previous work has shown that molecularly imprinted nanoparticles (MINPs) can play this role in standard 2D and 3D cell cultures, combining high recognition specificity with stability and cost-effectiveness. Taking this concept a step forward, here we prepared and tested MINPs against transforming growth factor (TGF)-β3, a regulator of stem cell tenogenesis, in hydrogel systems with bioinspired ordered microstructures. Our hypothesis is that combined control over biophysical and biochemical cues will synergistically contribute to more robust tenogenic commitment of stem cells. An N-terminal epitope of TGF-β3 was used as template molecule for imprinting MINPs by solid phase polymerization of acryloyl-containing monomers. MINP affinity was assessed by surface plasmon resonance (SPR), while selectivity was evaluated by Western blot after incubation in platelet lysate. Aligned polycaprolactone (PCL) meshes were first produced by electrospinning, followed by cryosectioning into 50 μm microfibers. To enable the efficient remote orientation of microfibers within hydrogels, superparamagnetic iron oxide nanoparticles were synthesized by thermal decomposition method and incorporated in the electrospinning solution. Finally, tenogenic constructs were prepared by encapsulating human adipose tissue-derived stem cells (hASCs), along with microfibers and MINPs, in transglutaminase-crosslinked gelatin hydrogels. Microfibers were unidirectionally aligned by applying a uniform magnetic field during gelation. SPR results demonstrated a remarkable affinity of MINPs for the template (17.96±13.19 nM), in the range of some monoclonal

antibodies. This compares favorably to the negligible interaction observed between the TGF-β3 epitope and MINPs imprinted against biotin, demonstrating the impact of the imprinting process on the molecular recognition potential of these nanoparticles. Magnetic microfibers easily formed homogeneous dispersions, allowing their incorporation and remote orientation in gelatin hydrogels. Viable hASCs could be cultured for at least 14 days, showing a preferential orientation along the microfiber alignment axis. MINPs have also been successfully included in these constructs, thereby combining specific biochemical and biophysical cues conductive to tenogenesis. Gene expression and protein synthesis are currently being analyzed to determine the phenotypic outcome of these constructs.

Acknowledgements: EU HORIZON 2020 for project ACHILLES (H2020-WIDESPREAD-05-2017-Twinning 810850) and ERC CoG MagTendon (ERC-2017-CoG-772817); FCT/MCTES for scholarship PD/BD/143039/2018 under Doctoral Programme PATH (PD/00169/2013), for project SmarTendon (PTDC/NAN-MAT/30595/2017), and contract 2020.03410.CEECIND. Authors declare no conflicts of interest.

P03
SILK-BASED 3D-PRINTED SCAFFOLDS TO TREAT MENISCUS DEFECTS

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Meniscus is one of the most injured tissues in orthopedics that require surgical care. Saving the meniscus is the initial intention, and repairing it with sutures is not always feasible. If the suturing of the meniscus is not possible, substitution of the meniscus with scaffolds is prefered considering the indications and contra-indications [1, 2]. Silk fibroin is a commonly used biomaterial in tissue engineering. It is known that the regenerated silk fibroin scaffolds support cell culture, they are not suturable, therefore not suitable for meniscus applications. In the present study, we manufactured and characterized scaffolds made of regenerated silk fibroin, which is reinforced, with 3D-printed mesh from polycaprolactone in the middle. The results of this study indicated that the developed reinforced scaffolds had the suture retention strength up to four times as that of the scaffolds without reinforcement, while up to five times regarding the water uptake capacity. To characterize the biocompatibility of the scaffolds *in vivo* that were either seeded with meniscus cells or human Hoffa's fat pad-derived stem cells, a subcutaneous implantation model in mice was performed. The micro-structure of the explants was analyzed by micro-CT. The histological study of the explants showed that good tissue infiltration, and it was observed those new blood vessels were formed within the scaffold. The results are encouraging to study further the in vivo performance in a large animal model.

Acknowledgments: The authors thank the financial support under the Norte2020 project (NORTE-08-5369-FSE000044) and the Portuguese Foundation for Science and Technology (FCT) program (PD/BD/143081/2018). IFC thanks the TERM RES-Hub, Tissue Engineering and Regenerative Medicine Infrastructure project, funded by FCT. The FCT distinction attributed to JMO under the Investigator FCT program (number IF/01285/2015) is also greatly acknowledged.

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P04
MICROFLUIDIC-CONTROLLED INTERFACIAL COMPLEXATION OF COLLAGEN AND GLYCOSAMINOGLYCANS

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Electrostatic complexation can be defined as a self-association of oppositely charged entities, *e.g.* polyelectrolytes. Such complexation can be performed in aqueous environments and does not require any modification of the used charged entities. Thus, it is an attractive approach in biomedical engineering because charged biomolecules with preserved bioactivity can be used. The generated complexes are versatile building blocks that can be processed into diverse biofunctional configurations. For example, fibrillar structures can be produced by interfacial polyelectrolyte complexation (IPC) - a complexation occurring at the interface of solutions of polymers with opposite charges [1]. However, controlling IPC is challenging, making difficult the production of homogeneous and continuous fibers. We hypothesize that these difficulties can be mitigated using microfluidic devices that transport polyelectrolytes side-by-side under a laminar flow. We used charged biopolymers that are major components of the extracellular matrix: collagen type I (Col) as a polycation and a glycosaminoglycan - heparin (Hep) or chondroitin sulfate (CS) - as a polyanion. Col was injected through a central channel, and the glycosaminoglycan through two converging lateral channels. The formation of IPC at the boundary between the biopolymers' streams was confirmed by phase-contrast microscopy. Microfibers were obtained by continuously pulling this complex from the microfluidic outlet. Upon contact with air, a quick solvent evaporation occurs resulting in the formation of a microfiber. We were able to handle and knit the dry microfibers without damage. SEM and bright-field microscopy confirmed the homogeneity of the microfibers. Polarized light microscopy suggests that they are composed of adjacent, aligned nanofiber bundles. The dimensions of the microfibers in aqueous solutions were affected by the used glycosaminoglycans: diameters of about 200 μm and 370 μm were measured for Col/Hep and Col/CS microfibers, respectively. This difference is due to the stronger negative charge of Hep when compared to CS, which results in the formation of a more compact IPC when Hep is used. Col/Hep microfibers also have higher tensile strength - about 5 kPa, compared

to 0.8 kPa for Col/Hep. Col, CS and Hep are abundant components in the ECM of the central nervous system. We envisage that the developed fibers can be used to promote tendon cells alignment along the nano- and microfiber pattern, *i.e.* as scaffolds for tendon regeneration.

Acknowledgments: Portuguese FCT (grants CEECIND/02842/2017, CEECIND/00352/2017, SFRH/BPD/85790/2012, projects PTDC/BTM-MAT/28327/2017, PTDC/BTM-ORG/28070/2017, PTDC/BTM-ORG/28168/2017, PTDC/NAN-MAT/28468/2017, PTDC/CTM-REF/0022/2020), European Commission (project H2020-WIDESPREAD-2014-668983).

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P05
DENDRIMERS CONJUGATED WITH MANGANESE FOR ISCHEMIC STROKE IMAGING

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Stroke survivors suffer numerous impairments, as paralysis and disfunctions in basic functions, affecting their independence and consequently their quality of life [1]. Existing treatments provide a narrow window of treatment and the recovery of function is still limited [2]. The repair of the CNS can be achieved by helping/treating surviving cells and by inducing endogenous repair processes. The present work consists of the production of carboxymethylchitosan/poly(amidoamine) (CMCht/PAMAM) dendrimers [3] loaded with manganese (Mn^{2+}) that will allow a continuous monitorization of the upon transplantation, through manganese-based MRI imaging. Results demonstrate a successful conjugation of the CMCht-PAMAM with the Mn^{2+}, as demonstrated by the ^{1}NMR analysis, which shows new peaks on the modified CMCht-PAMAM upon manganese introduction. The DLS analysis further demonstrated that the size of the produced PAMAM increased, and the zeta potential passed from negative to neural, which is related to the negative charge of the dendrimers together with the positive charge of the manganese. Cytotoxicity assay showed the CMCht-PAMAM+Mn^{2+} do not produce a toxic effect on the human Mesenchymal Stem Cells (hASCs) and are easily internalized (after 24 hours of culture the cells presented 99% of internalization). MRI imaging of the CMCht-PAMAM+Mn^{2+} showed the dendrimers' signal is detectable (in T1 weighted MRI) at 0.5 mg/ml, with a signal equivalent to the manganese solution control. All these results demonstrate that the produced dendrimers do not produce a toxic effect at a concentration that is detectable for MRI imaging, making them an appealing strategy for MRI imaging of both materials and cells upon transplantation.

Acknowledgements: This work is supported by the project 2IQBIONEURO (0624_2IQBIONEURO_6_E) and E. P. Oliveira PhD scholarship (SFRH/BD/37726/2018/J621340zkMFP) from the Portuguese Foundation for Science and Technology (FCT) and through JUSThera project (NORTE-01-0145-FEDER-000055). This work also acknowledges FCT for the JMO (IF/01285/2015) and the JSC (IF/00115/2015) distinction attributed under the program Investigador FCT.

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P06
HEMOCOMPATIBILITY OF POSITIVELY AND NEGATIVELY CHARGED NANOPATTERNED COATINGS

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In tissue engineering and regenerative medicine, implants are usually used to replace a missing biological structure or support the healing of damaged tissue. Upon implantation, the contact between the blood components and these foreign devices can cause different complications. Among these, the most common issue is the blood coagulation and consecutive thrombi formation that obstructs the normal blood flow. Therefore, a major requirements for any blood-contact devices are anti-thrombogenicity and low hemolysis. Herein, we present the self-assembly of poly(styrene-block-2-vinylpyridine) (PS-b-P2VP) block copolymers as a strategy to generate thin coatings with anticoagulant and antithrombotic properties. Using copolymers with different molecular weights and different solvents (chloroform or toluene) for vapor annealing, we generated coatings with different morphology and surface chemistry. P2VP quaternization allowed the introduction of a positive charge and the posterior modification of the assembled coatings with heparin. We found that the antithrombotic and hemocompatible properties depend on the coatings' chemistry and morphology: quaternized and heparinized coatings assembled from longer blocks presented excellent properties and are thus, suitable for coating implantable devices.

Acknowledgments: Portuguese Foundation for Science and Technology for the grants PTDC/QUI-POL/28117/2017, PD/BD/128085/2016, and CEECIND/00814/2017.

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P07

3D TENDON-ON-CHIP MODEL TO INTERROGATE THE MULTICELLULAR CROSSTALK IN HEALTHY AND DISEASED TENDON

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Different cell populations of tendon tissue play important roles on tendon physiology and pathophysiology. A better understanding of their complex cellular environment and crosstalk is critical for decoding the healing mechanisms in tendon injuries and to find new therapeutic options. However, the current lack of representative tendinopathy models is a major barrier for the progress of this field. The inflammatory phase of tendinopathy is characterized by increased vascularization and influx of immune cells (mast cells, macrophages, T cells) at the healing site. The aim of this work is to establish a multicellular organotypic 3D model recreating key signaling hallmarks of that immune response. We propose here a 3D compartmentalized tendon-on-chip composed by 3 fundamental components: 1) microenginnered tendon stroma that recapitulates the microstructural features of healthy and diseased (fibrotic) tendon; 2) perfusable channels representing the existing vasculature of the extrinsic tendon compartment; and 3) the circulating or tissue resident immune cells of interest. In a first setting, this dynamic system will be applied for studying the auto-regulatory feedback loop existing between T cells and tenocytes in tendinopathy. A microfluidic chip consisting of one central chamber and 2 side channel design was used as platform to build this adaptive/stromal interface. Hydrogel encapsulated human tendon derived cells (hTDCs) were loaded in the central chamber while microvascular endothelial cells successfully endothelialized the perfused side microfluidic channels. To recapitulate the different biophysical cues of a healthy and diseased tendon ECM microstructure, magnetic polycaprolactone (PCL)-based microfibers were incorporated within gelatin or platelet lysate (PL) hydrogels and their degree of alignment on chip was controlled by external magnetic fields. The resulting topographical cues revealed to be effective on controlling 3D cell organization and *de novo* matrix deposition, particularly in the case of PL-based hydrogels. Changes on the expression of genes and proteins related with

ECM, tenogenic markers and inflammatory signaling pathways are being evaluated. Circulating T cells will be next incorporated in this physiomimetic system in order to study the effect of hTDCs on their migration and activation, as well as the impact of these crosstalk mechanisms on the stromal compartment. Moreover, the magnetic responsiveness of the proposed system opens the perspective to further access the immunomodulatory potential of magnetic biomaterial/scaffolds, not only laying groundwork for better understanding tendinopathy, but also for tendon tissue regeneration and repair.

Acknowledgments: ERC Grant 772817; FCT for PD/BD/129403/2017, contract CEECIND/01375/2017, 2020.03410.CEECIND and PTDC/NAN-MAT/30595/2017. ED481B2019/025.

ACHILLES
ADVANCING TENDON REGENERATIVE THERAPIES

P08
DIRECT BIOPRINTING OF 3D HUMAN TENDON MODELS EMBEDDED ON A BIOMIMETIC FIBRILLAR MATRIX PLATFORM

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Tendon pathologies are highly debilitating and current treatment options have slow recovery rates, resulting in long rehabilitation periods and patient suffering. As such, relevant in vitro models that allow the study of these pathologies and test new regenerative approaches to develop better treatments are highly needed. 3D Bioprinted microphysiological systems (MPS) promises improved predictive power over traditional Petri dishes cell cultures due to their potential to create more biomimetic cell patterns and enabling process automation for reproducible sample replication. However, commonly used macromolecular bioink polymers have limited ability to mimic a structure as rich as tendon whose extracellular matrix (ECM) is responsible for activating and regulating several signaling pathways controlling cell behavior. Bioinks based on decellularized ECM (dECM) have emerged in recent years as an alternative biomaterial that better represent the signaling complexity of their native microenvironments. Following these concepts, here we successfully decellularized porcine flexor tendons in order to create a bioink that closely recapitulate the biophysical and biochemical cues of tendon cell niche and thus can self-induce the tenogenic differentiation of stem cell. For the feasible fabrication of human MPS, we used easily accessible human adipose derived stem cells (hASCs). The bioink was directly printed within cellulose nanocrystals (CNC) fluid gels used as support media for freeform bioprinting of embedded constructs with the desired 3D patterns. The subsequent induction of CNC self-assembly post-printing results in a permissive ECM mimetic fibrillar material housing the printed MPS with controlled cell organization and compartmentalization, and holding structural stability to support long-term in vitro cell maturation. This system showed high cell viability, proliferation, and alignment during culture up to 11 days, demonstrating that the synergy between dECM cues and printed patterns induce cells organization similar to tendons tissues. Gene and protein expression assays are currently being performed to characterize cells phenotype. Overall, the result obtained so far suggest that the proposed system might be promising

for the automated fabrication of organotypic tendon-on-chip models that will allow to study tendon physiology and pathologies or the effect of drugs for the treatment of tendinopathy.

Acknowledgements: EU HORIZON 2020 for project ACHILLES (H2020-WIDESPREAD-05-2017-Twinning Grant No. 810850) and ERC CoG MagTendon (ERC-2017-CoG-772817); FCT/MCTES for project SmarTendon (PTDC/NAN-MAT/30595/2017), FCT for the PhD grant of SMB (PD/BD/129403/2017) and contract 2020.03410.CEECIND.

P09
EX-VIVO STATIC CULTURE SYSTEM TO UNDERSTAND THE MECHANICS OF CUTANEOUS SCARRING

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Inflammatory diseases have been the focus of several clinical studies due to the limitations and several effects of currently available therapies. Therefore, there is an urgent need for safe, effective, new, and easily administrated therapies. Plants are a huge resource of bioactive compounds. Therefore, in this study, we investigated the immunomodulatory effects of aqueous (AE), ethanolic (EE) and dichloromethan (DE) extracts obtained from flowers, leaves and roots of *Echinacea purpurea* using LPS-stimulated macrophages (THP-1 cell line) as an *in vitro* inflammatory model. The *Echinacea purpurea* extracts were cytocompatible, since the cells metabolic activity, DNA concentration and phenotype were not affected. Under an inflammatory scenario, all the *Echinacea purpurea* extracts drastically reduced the pro-inflammatory cytokines and the reactive oxygen and nitrogen species (ROS/RNS), having DE the strongest anti-inflammatory compounds. Moreover, in the experimental conditions used in this study, *Echinacea purpurea* extracts showed generally more robust anti-inflammatory activity than the well-known NSAIDs, namely dexamethasone, diclofenac, salicylic acid and celecoxib. Besides anti-inflammatory activity, AE were also able to stimulate macrophages to produce pro-inflammatory cytokines, in a concentration dependent manner. Therefore, *Echinacea purpurea* extracts can be used to isolate new drugs, enabling treating disorders of the immune system, such as auto-immune diseases or cancer.

Acknowledgements: We acknowledge the financial support by the FCT to the PhD grant of SV (PD/BD/135246/2017) and the projects PATH (NORTE-08-5369-FSE-000037), SPARTAN (PTDC/CTM-BIO/4388/2014), Flexpol (H2020-NMBP-PILOTS-2016 721062) and FROnTHERA (NORTE-01-0145-FEDER-000023).

P10
SIGNATURES OF DERMAL EXTRACELLULAR MATRIX OF DYSTROPHIC EPIDERMOLYSIS BULLOSA PATIENTS

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Mutations in the *COL7A1* gene, which encodes collagen VII protein, the major component of the anchoring fibrils in the dermal-epidermal junction, cause all forms of dystrophic epidermolysis bullosa (DEB). Different clinical variants have been described with both dominant and recessive inheritance. However, information regarding the consequences of different *COL7A1* mutations in the cell microenvironment, particularly on extracellular matrix (ECM), is still scarce. Moreover, several studies found the spectrum of biologic and clinical phenotypes of DEB to be wider than initially anticipated. Hence, this work aims to unravel the main differences in the composition of the ECM of the dermis of DEB patients depicting different variants of the disease. For that purpose, we used cell sheet engineering to mimic the biological nature of the dermal compartment in normal and pathologically altered skin. Healthy primary fibroblasts and immortalized cell lines of three DEB variants (representing different aggressiveness degrees of the disease), provided by EB house Austria, were cultured for 14 days with ascorbic acid in order to promote maximum ECM deposition. Mass spectrometry-based label-free quantification was used to assess changes in the ECM deposited by the different cell populations. Then a combination of western blot, quantitative real-time PCR and histological methods were used to confirm the proteomic results and investigate the associated biological pathways. Analysis of the extracellular proteome revealed that fibroblasts from each DEB variant have their own proteomic signature. Independently of the DEB variant - and its associated clinical aggressiveness - the different *COL7A1* mutations studied impacted dermal ECM organization through the down-regulation of major ECM players such as collagen XII, decorin, biglycan and fibulin-5. Furthermore, ECM organization-associated proteins

were found to be differently expressed between DEB variants. For the phenotypes associated to increased severity of disease, a down-regulation of proteins linked to ECM structure and remodelling, namely collagens I, III and V and matrix metalloproteinases 1 and 2, was observed. Our results corroborate previous studies showing that total loss of collagen VII has an enormous impact on dermal ECM dynamics. Additionally, our results also demonstrated that a partial loss of type VII collagen impacts cell microenvironment, affecting mostly the ECM structural proteins. Overall, our work contributes to the generation of further knowledge on DEB variants molecular features.

Acknowledgements: The authors would like to acknowledge FCT for grant SFRH/BD/137766/2018(MDM) and contract CEECIND/00695/2017(MTC), the ERC Consolidator Grant – ECM_INK(ERC-2016-COG-726061) the European Union for The Discoveries Centre for Regenerative and Precision Medicine(H2020-WIDESPREAD-2014-1-739572).

P11

PLATELET-DERIVED EXTRACELLULAR VESICLES SHOW THERAPEUTIC EFFECTS ON A 3D TENDON DISEASE MODEL

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Tendon diseases are common clinical problems that can dramatically affect the quality of life of individuals across the demographic spectrum. Current clinical approaches do not tackle the etiology of the disease, underlined by an unresolved inflammatory scenario that provokes hypercellularity, neovascularization, and a dysregulation of the critical balance between extracellular matrix (ECM) remodelling proteases and their inhibitors. This scenario alters native ECM components and organization (loss of collagen anisotropic organization), a typical characteristic of scar tissue, resulting in reduced biomechanical strength, resulting in higher risk of acute tendon rupture. Extracellular vesicles (EVs), a diverse group of small membrane-enclosed particles actively released by all types of cells with key roles in cell communication, are very attractive as therapeutic agents to trigger repair/regenerative processes in injured tissues. Herein, we aimed to evaluate the therapeutic potential of small EVs (sEVs) and medium EVs (mEVs) derived from platelet lysate (PL) in a disease tendon-like *in vitro* model. The bioengineered 3D tendon disease model consisted of electrospun anisotropic fibrous scaffolds coated with human tendon-derived cells (hTDCs) encapsulated in PL as provisional ECM. Then, PL-derived EVs isolated by differential centrifugation were added to the hTDCs culture media and assessed the influence of EVs in tendon cells phenotype and ECM remodeling. After 14 days of culture of tendon cells on the anisotropic fibers, cells presented a disease-like phenotype, as previously shown [1]. We showed that EVs reestablish the expression of tendon-related markers like *MKX*, *SCX*, and *TNMD* in diseased hTDCs. Moreover, EVs increased the expression of different ECM components such as *COL31A* and *DCN*, and the expression of *MMP-3* and *TIMP-1,* which control the balance between the synthesis and degradation of tendon ECM. We also observed that EVs modulate the immunomodulatory response by increasing the release of anti-inflammatory mediators, e.g., IL-4, which might contribute to promote the resolution of inflammation of damaged tissue. Overall, we showed that PL-derived EVs have a positive influence on tendon cells cultured on a disease-like *in vitro* model, increasing the expression of healthy tendon cells markers,

promoting ECM remodelling, and increasing the expression of anti-inflammatory cytokines. In conclusion, EVs might be a promising therapeutic tool for tendon injuries recovery.

Acknowledgements: ERC CoG MagTendon grant agreement 772817; EC Twinning project Achilles 810850; FCT for PhD grant PD/59/2013 and PD/BD/135255/2017, Post-Doc grant SFRH/BPD/112459/2015, CEECIND/01375/2017.

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P12
EXPLORING EXTRACELLULAR MATRIX FEATURES OF PEMPHIGUS VULGARIS DISEASED SKIN CELLS

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Pemphigus vulgaris (PV) is a mucocutaneous autoimmune blistering disease caused by autoantibodies against desmosomal adhesion proteins. The most well-known autoantigens are Desmoglein 1 (DSG1) and 3 (DSG3). The targeting of these molecules results in the loss of cohesion between suprabasal keratinocytes in the epidermis in a process known as acantholysis. The efforts of the scientific community to unravel the roots of PV have predominantly been focused on the affected keratinocytes, leaving a possible role for fibroblasts adjacent to the blister region still in the open. To explore this possibility, we isolated fibroblasts from diseased skin samples from PV patients and from healthy donors. We cultured the isolated fibroblasts with ascorbic acid supplementation to stimulate the deposition of extracellular matrix (ECM). After 14 days of culture we analyzed the generated cells sheets and observed that although resistant to manipulation, cell sheets obtained from diseased cells were noticeably thinner and more prone to tear than those derived from healthy fibroblasts. In order to characterize the deposited ECM we used Biocolor kits to quantify collagen, elastin and glycosaminoglycan content and observed that these are diminished in cell sheets from diseased fibroblasts. Additionally, protein expression analysis showed that cell sheets from diseased fibroblasts have decreased levels of Collagen 1, a key structural component of ECM, Fibulin-5, key in the development of elastic fibers and 67LR, an important molecule in cell adhesion to the basement membrane. Together, these results show that fibroblasts from the region surrounding the skin blisters are also affected in their structural components, highlighting a potential role for these cells in the etiology of PV that is worth of further investigation.

Acknowledgements: ERC Consolidator Grant – ECM_INK (ERC-2016-COG-726061).

P13
POLYELECTROLYTE COMPLEXATION OF CATECHOL CONTAINING POLYSACCHARIDES FOR THE DEVELOPMENT OF BIOADHESIVE MEMBRANES

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Mussels secrete protein-based byssal threads to tether to rocks, ships, and other organisms underwater [1]. The secreted marine mussel adhesive proteins (MAPs) contain an unusual amino acid L-3,4-dihydroxyphenylalanine (DOPA). The catechol groups of DOPA and its analog, dopamine (DN), are major contributors for these adhesive properties and have motivated the development of (bio)adhesive materials via their conjugation to different polymers [2]. We hypothesize that polyelectrolyte complexation (PEC) of catechol-modified polysaccharides can be used to obtain adhesive membranes. PEC is a versatile approach for assembly of biofunctional and biocompatible materials. This is a flexible production method since the formation of polyelectrolyte complexes (PECs) results simply from electrostatic interactions between oppositely charged entities. Herein, we used two natural and biocompatible polymers: chitosan (CHI) as a polycation and hyaluronic acid (HA) as a polyanion [3]. Combinations of these polymers modified with catechol groups were made to enhance the adhesive properties of the assembled membranes [4]. Preliminary lap shear stress tests showed promising adhesiveness from membranes that contain catechol-modified polymers. Thus, we envisage adhesives suitable for the regeneration of soft and hard tissues, such as in the tendon-bone interface.

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P14

IONIC LIQUID-ASSISTED SYNTHESIS OF POROUS SAIB/SILK FIBROIN SCAFFOLDS FOR BIOMEDICAL APPLICATIONS

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Sucrose acetate isobutyrate (SAIB) is a viscous liquid with several processing challenges when envisioned as a tissue engineering product. SAIB is traditionally allocated to food and cosmetic activities, usually by dissolving it in oils/waxes. SAIB application in the biomedical field is stringently associated with controlled drug release from the settled depots, inevitably requiring the use of organic solvents that injure nature and health [1]. Thus, the authors hypothesized that using ionic liquids as solvents, a new methodology of processing SAIB could emerge to avoid the use of organic solvents and to favor biocompatible scaffolds for tissue engineering applications. Ionic liquids (ILs) have outstanding thermal and chemical stability, low flammability, substantial task specificity, narrow vapor pressure, minimal environmental release, and low melting points [2]. In this work, we propose a novel method to develop SAIB/SF scaffolds by processing SAIB combined with silk fibroin (SF) with ionic liquids (ILs). The presence of SF and SAIB was observed by Fourier Transform Infrared technique, which also showed that the IL was effectively removed. The characterization made to the scaffolds also shown a mean values of porosity (88 ± 3)%, pore size of (135 ± 17) μm, and interconnectivity of (95 ± 3)% (observed by Scanning electron microscopy and determined by Micro-computed tomography analyses). The mechanical spectra show a predominant gel character, with storage modulus (G'/Pa) higher than the loss modulus (G''/Pa). The produced scaffolds could also support the adhesion of (0.26 ± 0.03) N.s. Furthermore, the experiments with fibroblast cell line - L929 and human adipose-derived stem cells (hADSCs) revealed that the scaffolds could also support living cells when cultured in scaffolds extracts or in direct contact with cells, respectively, exhibiting exceptional cytocompatibility and cell viability. Lastly, the hemolysis test showed that the scaffolds had lower than 2% hemolytic rates, which indicates blood biocompatibility. Overall, the collected findings showed that the produced SAIB/SF scaffolds have a great potential for tissue engineering scaffolding applications.

Acknowledgments: The authors acknowledge the financial support from "Fundação para a Ciência e Tecnologia" Portugal (SFRH/BPD/100957/2014, SFRH/BPD/94277/2013 - CTTI-58/18-I3Bs(4), CEECIND/00111/2017, PD/BD/135247/2017, CTTI-57/18-I3BS(5), and CEECIND/01306/2018) and the financed project PTDC/BTMMAT/29760/2017 (POCI-01-0145-FEDER-029760), by FCT and co-financed by FEDER and POCI.

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P15
SYNTHESIS AND CHARACTERIZATION OF NEW QUATERNARY BIOACTIVE GLASS NANOPARTICLES FOR ORTHOPEDIC APPLICATIONS

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Bioactive glass (BG) as well as bioactive ceramics, are promising materials for tissue engineering due to their controlled degradability and capability to stimulate new tissue formation [1]. BGs are especially attractive for orthopedic applications, since it forms strong bonds with the bone through the formation of hydroxyapatite (HA) resulting from the release of ions like Si, Ca and P, that stimulates the formation of bone tissue when implanted in the living body [2]. Particularly, the resulting apatite layer mimics chemical and structurally the mineral phase of bone [3]. Sol-gel processing allows the production of bioactive glass nanoparticles (BGNPs) with distinct compositions and the incorporating of different metallic ions with therapeutic benefits into the glass network [4]. The addition of these ions into bioactive glasses can cause changes in the crystal structure, specific surface, thermal stability, morphology, solubility, and chemical and biological properties. These trace elements have been found to play crucial roles in the formation, growth, and repair of the bone [5]. Moreover, bioactive glasses (BG) doped with small amounts of silver ions showed a broad spectrum of antimicrobial activity. Low concentrations of silver ions in BG are not toxic, but high concentrations can cause cytotoxicity. Due to the antimicrobial properties of silver, the recent focus on the development of silver-doped implants is increasing [5], [6]. On the other hand, strontium (Sr) is an important element of the human body that has a significant influence on bone metabolism. Recent studies have revealed that a low dose of strontium ion stimulates bone formation and osteoblast replication while promoting inhibition of bone resorption by osteoclasts [3], [6]. Attending that the functionality of produced nanoparticles is directly dependent on the doping elements [3], herein the effect of the doping on the properties of synthesized nanoparticles was analyzed through some characterization techniques as FTIR, SEM-EDS and Zeta potential, and also *in vitro* bioactivity studies were conducted.

Acknowledgments: Portuguese Foundation for Science and Technology (FCT) and European program FEDER/FEEI through projects PTDC/NAN-MAT/31036/2017 and PTDC/BTM-MAT/28123/2017.

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P16
SUPRAMOLECULAR HYDROGELS INDUCE DIFFERENTIATION OF STEM CELL INTO NEURAL LINEAGES

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Peptide-based supramolecular hydrogels have been proposed as supports for tissue engineering. [1] They are generated by the supramolecular organization of bioactive peptide amphiphiles (*i.e.*, building blocks) into nanofibres, that are maintained together through several non-covalent interactions, *e.g.*, p-p staking, and H-bonding. These nanofibers can further form hydrogels by interactions with ions from the cellular milieu. The obtained hydrogels are dynamic and sensitive to different stimulus: changes in temperature, pH or ionic strength can trigger their assembly/disassembly or gelation/solubilization. [2] The peptide can be functionalized with carbohydrates to copycat bioactive glycoproteins present in the extracellular matrix (ECM). Herein, we hypothesized that short self-assembling glycodipeptides can be used as building blocks of supramolecular gels that induce stem cell differentiation. [2] Our molecular design is based on the Fmoc-diphenylalanine (Fmoc-FF) known to generate supramolecular hydrogels under physiological conditions. Fmoc-FF was functionalized with glucosamine-6-sulfate (GlcN6S) - a structural element of several glycosaminoglycans known for their ability to modulate cellular behavior via interactions with numerous proteins. [3] The gelation of Fmoc-FF-GlcN6S in response to temperature change (T, *i.e.*, a heating-cooling cycle) and solvent-switch (S, dilution of a DMSO solution with water) was studied. Both methods generated hydrogels that were stable for at least 21 days under cell culture conditions. Importantly, the preparation method influenced the stiffness of the hydrogels: at the same concentration of Fmoc-FF-GlcN6S (10 mM), gels obtained by method T had a Young's modulus of 2.1 kPa, while S method gels had a modulus of 0.5 kPa (in the range of neural tissues, *i.e.*, between 0.5-1.9kPa). CD, fluorescence and AFM data showed that this difference is due to different molecular packing and nanofiber morphology. Both types of hydrogels were cytocompatible with adherent adipose-derived stem cells (ADSC), that overexpressed neural genetic markers, such as GFAP (glial fibrillary acidic protein) and Nestin (neuronal stem cell maker) after three days of culture. Longer cultures (nine days) overexpressed the microtubule associated proteins MAP2 and βIII-tubulin. These qPCR results were also

confirmed by immunofluorescence. In conclusion, the developed Fmoc-FF-GlcN6S hydrogels induce stem cell differentiation into neural cell lineages and present the conditions to be tested for the regeneration of neural tissues.

Acknowledgments: We acknowledge the financial support from the EC (#668983-FORECAST) and FCT (PhD grant PD/BD/135256/2017).

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P17
END-ON CONJUGATION OF GLYCOSAMINOGLYCANS TO LINEAR POLYMERS FOR THE PREPARATION OF ECM-LIKE HYDROGELS

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Proteoglycans (PGs) are brush-like molecules present in the extracellular matrix (ECM) comprised by glycosaminoglycans (GAGs) attached by its reducing end to a protein core. They play structural and regulatory roles in the ECM and are involved in cellular signaling processes governing tissue growth and development. In tendon, the PG decorin, biglycan, fibromodulin and lumican bind to collagen fibrils and actively participate in their assembly (i.e. fibrillogenesis) and therefore in tendon function. Additionally, PGs from lectican family, aggrecan and versican, bind to HA and collagen and the formed complex sustains the high compressive strength in this tissue [1]. Simultaneously, PGs are reservoirs for growth factors, chemokines and cytokines, protecting them from enzymatic degradation, or releasing them to trigger cell-signaling pathways [2]. Because of these roles, PGs are crucial in the regeneration of tendon tissue, among others, and therefore hold a high potential as matrixes for tissue engineering and regenerative medicine. Because PGs isolation from animal tissues is challenging, the common strategy to prepare hydrogel matrixes for regeneration therapies is the covalent crosslinking of one or several ECM components, or the use of glycopolymers that mimic PGs [3]. However, the generated gels do not mirror the real organization of the ECM and hinder the specific interactions of GAGs with other ECM proteins that are vital for cell-matrix crosstalk [3]. Herein, we synthetized brush-like copolymers that are close mimics of the natural PGs because high molecular weight GAGs were conjugated via their reducing end to linear polymers. Polyglutamic acidwas used as core,and GAGs such as chondroitin sulfate were conjugated by thiol-maleimide chemistry, a click reaction known to be highly effective, idealto achieve the highest possible brush density. Moreover,the linear polymer is functionalized at one of its ends with a HA-binding moiety that allows the following assembly with HA and collagen, mimicking with high precision the GAG organization and nanostructure of the native ECM. We believe that the resulting hydrogels can recreate the three-dimensional ECM-like microenvironment, holding great promise in the treatment of cartilage injuries and disorders.

Acknowledgments: The authors acknowledge FCT financial support (PD/BD/128085/2016, PTDC/QUI-POL/28117/2017, PTDC/BTM-MAT/28327/2017 and CEECIND/00814 /2017).

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P18

OPTIMIZATION OF KEFIRAN EXOPOLYSACCHARIDE EXTRACTION FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE APPLICATIONS

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Kefiran is an exopolysaccharide produced by the microflora of kefir grains that are used to obtain the health-promoting beverage kefir. The numerous beneficial properties associated to kefiran such as antimicrobial, antioxidant, antitumor, immunomodulatory, and anti-inflammatory properties, together with its physicochemical properties, have led to the exploration of this polysaccharide for numerous applications, mostly in the food industry and biomedical fields. The present work aimed to fully characterize and compare the kefiran polysaccharide obtained through different extraction methodologies to explore this biopolymer for tissue engineering and regenerative medicine (TERM) applications. High-quality kefiran polysaccharides with satisfactory yield were obtained through the different extraction protocols, and kefiran cryogels were successfully produced from the different extracts by freeze-drying. Both kefiran products, extracts and 3D scaffolds, were fully characterized for their structural and physicochemical properties through ^{1}H nuclear magnetic resonance spectroscopy (NMR), Fourier transform infrared spectroscopy (FTIR), gel permeation chromatography - size exclusion chromatography (GPC-SEC), differential scanning calorimetry (DSC), rheology, scanning electron microscopy (SEM) and micro-computed tomography (micro-CT). The biocompatibility of the different kefiran extracts and scaffolds was assessed by analysing L929 cell growth and proliferation through the AlamarBlue® cell viability assay (BioRad) and total double-stranded DNA (dsDNA) quantification. The different kefiran products obtained showed interesting structural, physicochemical, and biological properties revealing their biomedical potential and suitability for TERM applications.

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METHACRYLATED KEFIRAN HYDROGEL FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE APPLICATIONS

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Polysaccharides of microbial origin are a renewable resource that has been receiving increasing interest in tissue engineering and regenerative medicine (TERM) fields, especially to create new biomaterials. Kefiran is an exopolysaccharide produced by the microflora of kefir grains that has recently received particular attention for its biomedical applicability and Kefiran-based hydrogels have been successfully obtained through freeze gelation in previous research (1-3). Nevertheless, the application of these natural-based hydrogels is principally constrained by their fragile structure and poor mechanical properties, among other limitations. Thus, in order to overcome these shortcomings and enhance the polymer's properties, numerous cross-linking approaches are currently performed. In this study, through the chemical reaction of Kefiran with methacrylic anhydride (MA), a new methacrylated Kefiran product was obtained (MA-Kefiran) that was also successfully freeze-dried into 3D scaffolds. The obtained MA-Kefiran products were fully characterized, and their properties evaluated with several methodologies. FTIR and ^{1}H-NMR spectroscopies confirmed the occurrence of the chemical modification of MA-Kefiran. This research revealed that MA-Kefiran has a Mw of 800 kDa and pseudoplastic behavior and the obtained hydrogel revealed, through micro-CT, high porosity and thick pore walls, with a homogeneous structure. Finally, MA-Kefiran hydrogel showed no cytotoxic response and an ability to improve the viability of L929 cells. The inclusion of methacrylate groups in the Kefiran polysaccharide structure resulted in the development of a promising MA-Kefiran hydrogel with improved properties for TERM applications.

Acknowledgments: H. Radhouani and C. Gonçalves were supported by the Foundation for Science and Technology (FCT) from Portugal, with references CEECIND/00111/2017 and SFRH/BPD/94277/2013, respectively. S. Correia and this work were funded by the R&D Project KOAT - Kefiran exopolysaccharide: Promising biopolymer for use in regenerative medicine and tissue engineering (refs.

PTDC/BTMMAT/29760/2017 and POCI-01-0145-FEDER-029760), financed by FCT and co-financed by FEDER and POCI.

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P20
LIGNOCELLULOSIC COMPOUNDS WITH ANTIBACTERIAL PROPERTIES TARGETING ENVIRONMENTAL AND BIOMEDICAL SURFACES

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Conventionally, during treatment or recovery, patients are in contact with different surfaces that could be infected with a variety of pathogenic microbes. The presence of microorganisms could lead to major health problems. Currently, the prevention of microbial adhesion and biofilm formation is performed by using antimicrobial reagents, such as antibiotics, fungicides, antiviral drugs, and nonpharmaceutical chemicals. However, the extensive use of these compounds causes concern due to their potential for environmental pollution, and the development of microbial resistance. Due to the disadvantages of using these disinfection materials, it became necessary to develop environmentally friendly antimicrobial agents, that are capable to prevent microbial adhesion and proliferation on the surface of the material and reduce their negative effects [1] . Lignocellulosic biopolymers from natural fibers or biomass are promising candidates as antimicrobial agents since are from renewable sources, high available, low cost, and biodegradable. The lignocellulosic fibers are mainly composed of three biopolymers, being cellulose, hemicellulose, and lignin [1-2]. Lignocellulosic materials such as cellulose extracted from wood were combined with sodium alginate to produce bio-sponges showing good antimicrobial potential against *Bacillus subtilis*, *Escherichia coli,* and *Pseudomonas aeruginosa* [3]. These positive antibacterial results combined with good structural and mechanical properties indicate that those structures might be applied in the biomedical field, such as scaffolds for tissue engineering, as drug carriers, or for wound dressing applications. Softwood kraft lignin combined with poly(butylene succinate) by extrusion process, also revealed good antioxidant and antibacterial behavior against *Staphylococcus aureus* [4]. The composite materials have shown antibacterial antioxidant activity even at low concentrations of lignin, showing a reduction of the bacteria adhesion of about 90%. Due to the antibacterial and antioxidant characteristics obtained for the composites, it is proposed that these composite materials should be used for biomedical applications. Natural biopolymers from lignocellulosic sources such as cellulose, hemicellulose, and lignin, have shown

promising potential to be used as antimicrobial agents in environmental and health sectors.

Acknowledgments: The authors acknowledge the financial support through the industrial project WINECOMP, FCT Grant SFRH/BPD/100760/2014 and R&D&I Structured Project with reference NORTE-01-0145-FEDER-000021.

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P21
CONTACTLESS MODULATION OF MACROPHAGES INFLAMMATORY RESPONSES USING SPIONS-MIRNA COMPLEXES

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Nucleic acids are powerful materials to probe or program cell responses. Altered miRNAs expression has been associated to inflammatory diseases, thus miRNA-based strategies present new immunoregulatory possibilities for precision and personalized regenerative therapies. However, efficient and precise delivery systems are still a challenge for translatable RNA-based approaches. A major obstacle is to find carriers that overcome the RNA instability and enhance intracellular release. Magnetically-assisted strategies to tackle inflammation holds potential to modulate cell and tissue responses combining contactless control and tissue penetration for tracking, local retention, and real time monitoring. Despite superparamagnetic iron oxide nanoparticles (SPIONs)-based approaches enable precise delivery of bioactive agents and remote control, they have been scarcely explored for targeted delivery and cell programming. This work aims to magnetically deliver and study miRNA molecules in the modulation of macrophage (Mφ) responses by blocking a miRNA sequence (miR-155-5p), known to be overexpressed in inflammatory states, and consequently repressing inflammatory proteins. To achieve this goal, we firstly conjugated SPIONs with polyethylenimine (PEI) and miRNA molecules to form magnetically-responsive complexes (SPIONs-miRNA) via electrostatic complexation. Then, we investigated stationary (SMF) and pulsed-electromagnetic field (PEMF) using MagnefectNano and MagnetoTherapy devices, respectively, for the internalization and delivery of SPIONs-miRNA by magnetofection. Afterwards, we investigated Mφ behavior to SPIONs-miRNA complexes. Our results show that SPIONs/PEI complexes were successfully produced with 56±2nm, and a surface charge of 14.1±0.9mV. To select the magnetofection procedure and to characterize cellular uptake, SPIONs/PEI complexes were FITC-labeled (SPIONs/PEI/FITC) and iron amounts investigated from 22 to 528 pg/cell. The measurement of non-internalized SPIONs/PEI/FITC showed an improved cell uptake using SMF comparing to PEMF. The distribution of SPIONs/PEI/FITC in cell culture

was detected by Pearl's stain with the most promising outcomes within 22-88 pg iron/cell. Additionally, confocal microscopy confirmed the SPIONs/PEI/FITC intracellular location using 3D image reconstruction. The ratio of SPIONs/PEI-miRNA was also investigated and SPIONs/PEI as low as 40ng are effective for miRNA loading. Ongoing studies on the expression and production of inflammatory mediators will validate the effective concentrations of SPIONs-miRNA complexes in Mφ responses. The work combines contactless with high precision control aiming to reprogram Mφ inflammatory profiles, whose outcomes are expected to contribute to advanced targeted and guided Mφ communication favoring a pro-regenerative environment and contributing to improved healing outcomes.

Acknowledgements: ERC CoG MagTendon (No.772817), H2020 Twinning project Achilles (No. 810850), FCT under the Scientific Employment Stimulus-2020.01157.CEECIND, and Norte-01-0145-FEDER-02219015 supported by Norte Portugal Regional Operational Program (NORTE 2020). Doctoral Grant:SFRD/BD/144816/2019.

P22
THE INFLUENCE OF PORCINE KIDNEY-DERIVED EXTRACELLULAR MATRIX ON THE DIFFERENTIATION OF STEM CELLS

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Kidney diseases are currently a global public health problem affecting almost 10% of the population worldwide. [1] Aging together with relevant risk factors can lead to the gradual loss of kidney functions and the progress from acute kidney injury to chronic kidney disease is frequently observed. [2] The standard of care, dialysis, extrinsically replaces kidney function but does not prevent the disease progression and the development of comorbidities. Hence, the number of patients included in the waiting list for renal transplantation increases year after year. [1] Currently, innovative tissue engineering technologies are being developed in order to improve the regenerative potential of kidney. [3] In this work, we will study how a decellularized-based hydrogel can influence the differentiation of stem cells into different renal phenotypes, envisioning the development of an injectable kidney advanced therapy. Briefly, different stem cell types will be encapsulated in a decellularized kidney extracellular matrix (DKECM)-based hydrogel of porcine origin and its differentiation will be evaluated over time. Ultimately, we intend to evaluate if the DKECM retains the biochemical and biological cues to influence stem cell differentiation into renal specific phenotypes.

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Compete2020 funding program (Project Cells4_IDs PTDC/BTM-SAL/28882/2017) for the financial support through the project.

P23

APPROACHING MAGNETIC CELL SHEETS UNDER HYPOXIC AND INFLAMMATORY ENVIRONMENT TO STUDY IMMUNOMODULATORY POTENTIAL FOR TENDON THERAPIES

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Tissue environments are complex and finely regulated by a multitude of factors that guide cellular behavior. Environments enriched with inflammatory signals compromise tendon homeostasis and influence tissue degeneration. The level of oxygen to meet metabolic requirements can also affect local responses suggesting that hypoxia may control apoptosis, inflammatory mediators, and matrix production. Published works reported a modulatory effect of pulsed electromagnetic field (PEMF) over inflammatory cues expressed by human tendon cells (hTDCs) [1]. PEMF was also shown to reduce the effects provoked by hypoxia affecting the regulation of transcription factors and inflammatory cytokines. Thus, we propose to explore the role of hypoxia in the management of inflammatory profiles of hTDCs conditioned to inflammatory cues, and the influence of PEMF over hTDCs exposed to hypoxia using a magnetic cell sheet (magCSs) model, enabling close interactions between tendon cells-matrix, which was previously established by our group [2]. In this work we aimed to investigate the response of magCSs made of hTDCs and magnetic nanoparticles (MNPs) under a permanent well-array magnet. MagCSs were pre-exposed to hypoxic environments induced by different percentages of oxygen tension (1%, 2%) and times of exposure (1h,4h,6h). To study the effect of hypoxia in the expression of inflammatory cues, magCSs were treated with IL-1β, following previously established conditions [1], and further exposed to the hypoxic environments investigated. Finally, we assessed the influence of a PEMF with the parameters 5Hz,4mT and 50% duty cycle on IL-1β-treated-magCSs exposed to hypoxic environments. Our results show that hypoxia increases the inflammatory profile and hypoxia-inducible factors: HIF-1α and HIF-2α in IL-1β-treated-magCSs at gene and protein levels. Moreover, the gene expression of pro-inflammatory factors (*TNFα, IL-6,IL-8*) and of *HIF-1α* and *HIF-2α* was increased in IL-1β-treated-magCSs, independently of the time or oxygen tension. Nevertheless, when IL-1β-treated-magCSs exposed to hypoxia are PEMF-stimulated, there is a decrease in the

expression of pro-inflammatory genes and an increase in the anti-inflammatory (*IL-4, IL-10*) gene expression. Overall, PEMF modulates the response of magCSs exposed to hypoxia favoring the expression of anti-inflammatory genes, holding evidence for immunomodulatory properties, even when magCSs are exposed to adverse environmental conditions provided by low oxygen tensions and inflammatory rich factors.

Acknowledgements: ERC CoG MagTendon (No.772817), H2020 Twinning project Achilles (No.810850), MagTT PTDC/CTM-CTM/29930/2017 (POCI-01-0145-FEDER-29930), FCT under the Scientific Employment Stimulus-2020.01157.CEECIND, and NORTE-01-0145-FEDER-000021 supported by Norte Portugal Regional Operational Programme (NORTE 2020). Thanks to Hospital da Prelada (Porto, Portugal).

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P24

FABRICATION OF MAGNETIC MICELLES FOR DRUG DELIVERY AND CELLULAR PROGRAMMING AIMING AT TARGETED NANOTHERANOSTICS

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The translation and clinical applicability of advanced medical nanotechnologies pursuing multiple functionalities to meet biological requirements for improved diagnostics and treatments remains an unfulfilled demand. To meet these challenges, hybrid superparamagnetic iron oxide nanoparticle polymeric micelles (SPION@PMs) have recently emerged as multifunctional tools able to bring into play the advantages of both the polymeric micelles (PMs) and superparamagnetic iron oxide nanoparticles (SPIONs). While PMs are formed by self-assembly of amphiphilic polymers, and are composed of a hydrophobic core and a hydrophilic shell able to incorporate hydrophobic and hydrophilic cargo, respectively, SPIONs present unique magnetic properties rendering magnetic field guidance. Such combination in SPION@PMs allows biocompatibility, high stability, and the ability to accumulate within tissues for tracking and contactless control of multiple therapeutic agents for extra- or intra- cellular delivery. Although, SPION@PMs have been prominently investigated for cancer focusing on chemotherapy drugs delivery and MRI, they are excellent candidates for transporting drugs and bioactive molecules envisioning finely tuned regenerative medicine strategies. In this work, we aim to prepare and characterize SPION@PMs, assembled from hydrophobically modified natural polymer chitosan (CS) and SPIONs, to be exploited for incorporating hydrophobic drugs (e.g. anti-inflammatory drugs), and in the surface, cell instructive molecules as small nucleic acids with regulatory functions for (re)programing cell responses. CS was modified with palmitic acid to produce palmitic acid-grafted-chitosan using carbodiimide chemistry. Proton nuclear magnetic resonance and Fourier transform infrared spectroscopy were used to evaluate the degree of modification of CS. The critical micelle concentration of the amphiphilic polymer was determined using Nile Red as a fluorescent probe. SPION@PMs were prepared by a ultrasonication method and purified by centrifugation and magnetic separation. The magnetic micelles present size of 358±11nm, a polydispersity index of 0.15±0.02 and colloidal stability over 2 weeks evaluated by dynamic light scattering.

Moreover, they exhibit a positive zeta potential of 26.6±0.7mV. Scanning transmission electron microscope revealed a spherical morphology and the presence of SPIONs inside the nanomicelle. Moreover, L929 cells showed high cell metabolic activity in culture with the nanomicelles suggesting nanomicelle feasibility for cell-oriented approaches. Overall, the obtained results indicate that magnetic nanomicelles were successfully developed and can be further exploited as a dual platform for incorporating organic drugs and gene molecules envisioning safe, effective and clinically suitable vehicles for improved treatment outcomes.

Acknowledgments: ERC CoG MagTendon (772817), EC Twinning project Achilles (810850), Norte-01-0145-FEDER-02219015 supported by Norte Portugal Regional Operational Programme (NORTE 2020).

P25
MARINE ORIGIN MEMBRANES BASED ON CHITOSAN AND COLLAGEN

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The human body has a limited ability to regenerate after a severe injury. Tissue engineering (TE) has emerged as a versatile solution for the development of biomedical devices to re-establish normal body functions using biofunctional materials to fill the injury site and to provide mechanical support. So far, numerous natural materials have been proposed for TE applications, including chitosan, a natural polycationic polysaccharide, that is obtained by the deacetylation of chitin, a structural element found in the exoskeleton of crustaceans mainly crabs and shrimps. Chitosan is currently used for tissue repair and wound healing, however, has a low cellular affinity. This drawback can be overcome through the combination of chitosan with other polymers, such as collagen, one of the major components of the extracellular matrix. Collagen type I can be obtained from various animal sources, including fish by-products, which reduce the risk of diseases transmissions. Nevertheless, the main challenge of these promising systems is the lack of bioadhesion between the material and tissue i.e. the capacity of the material to bind the target tissue and reconnect the broken bonds reducing the risk of cracking. This work focus on the development of bioadhesive membranes from marine renewable biomaterials, namely chitosan and collagen extracted from fish skins. The superior adhesive membranes were produced modifying the collagen-chitosan membranes with catechol groups. The adhesive strength showed that the incorporation of catechol groups indeed led to a significant improvement of their adhesion properties. It was also shown that the presence of the catechol groups enhances cell attachment, viability and metabolic activity. These data suggest that the developed marine-origin membranes could be used in tissue engineering applications.

Acknowledgements: Portuguese Foundation for Science and Technology (FCT) and the European program FEDER/FEEI for the financial support through PTDC/BTM-MAT/28123/2017 and PTDC/NAN-MAT/31036/2017 projects. FCT is also

acknowledged for the PhD scholarship SFRH/BD/143209/2019 granted to Cátia Correia.

P26
INTERNAL AND EXTERNAL METAL-COATINGS TO PREVENT BACTERIAL ADHESION ON URETERAL STENTS

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Over the years, metal-based coatings have gained prominence due to their ability to impair biofilm formation on biomedical implants, with emphasis for silver- and zinc-based strategies, which concomitantly present intrinsic antimicrobial properties and biocompatibility. In urology, bacterial infection is one of the main drawbacks associated with the use of ureteral stents, limiting their therapeutic action and often representing an increase in healthcare costs. After stent placement in the ureter and upon the passage of urine, the deposition of ion and minerals emerges, forming anchor points where bacteria may adhere and develop. Since the urine has in-stent and out-of-stent flows, the biofilm formation can occur inside or outside the stent. Unlike most strategies that only have exterior coatings, we proposed the development of internal and external coatings on polyurethane-based stents, in order to create a biomedical material with more efficient antimicrobial properties. We performed a modification on the inner and out surfaces of polyurethane stents (7F Tecoflex, Nordson Medical), starting by surface activation with tin(II) chloride dihydrate (≥98%, Sigma-Aldrich), followed by a electroless plating of metals, namely, silver and zinc, separately. X-ray diffraction (XRD), contact-angle, scanning electron microscope (SEM), and energy-dispersive X-ray spectroscopy (EDS) analyses were performed to properly characterize the modified surfaces. Biocompatibility assays were carried out following ISO10993-5, using L929 mouse fibroblast (ATCC NCTC clone 929:CCL 1). To evaluate antimicrobial effects of coatings, the modified stents were incubated, separately, with *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 700698 (Methicillin-resistant strain*), Pseudomonas aeruginosa*-resistent ATCC 27853 and *Proteus mirabilis,* following an established biofilm formation protocol. The designed protocol was able to coat both internal and external surfaces of stents, as proved by SEM, XRD, contact-angle, and EDS analyses. Both coatings were efficient against *E. coli*, *P. aeruginosa* and *P. mirabilis*, exhibiting antibacterial activity by lowering the adherence of strains, without compromise L929 cells viability. Thus, the development of inner and out metal-based coatings bestowed

appropriate antimicrobial properties to ureteral stents, conferring advantages comparing with the standard stents on the market.

Acknowledgments: this work received funding from Foundation for Science and Tecnology (FCT), FCT Doctoral Program in Tissue Engineering, Regenerative Medicine and Stem Cells PD/BD/150478/2019

P27

THE ANTI-INFLAMMATORY ACTIVITY OF ECHINACEA PURPUREA EXTRACTS AND ITS FRACTIONS INVOLVES THE MODULATION OF THE P38/ERK PATHWAY IN HUMAN MACROPHAGES

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Inflammatory diseases have been the focus of many clinical studies due to the limitations and frequently severe side effects associated to the currently available therapies. Therefore, there is an urgent need for new, safer, more effective, and easy to administer therapies. Plants were over the history important sources of bioactive compounds and are still today a valid basis for the discovery of new medicines. In this work, we studied the mechanism of action of the anti-inflammatory activity of three *E. purpurea* extracts. Dichloromethanolic extracts (DE) obtained from roots or flowers and ethanolic extracts (EE) obtained from flowers were prepared using an Accelerated Solvent Extractor (ASE). These three *E. purpurea* extracts significantly reduced interleukin 6 (IL-6) and the reactive oxygen and nitrogen species (ROS/RNS) production in lipopolysaccharide (LPS)-stimulated primary human macrophages, through the downregulation of the phosphorylation of p38 and extracellular signal-regulated kinase 1/2 (ERK1/2), in the mitogen-activated protein kinase (MAPK) signaling pathway. No effect was observed in nuclear factor-kappa B (NF-κB) signaling pathway. Interestingly, *E. purpurea* extracts were also able to decrease the expression of cyclooxygenase 2 (COX-2). DE obtained from roots demonstrated the strongest anti-inflammatory activity,

followed by DE obtained from flowers and EE obtained from roots. In order to understand which bioactive compound or class of compounds (phenols vs. alkylamides) are responsible for the anti-inflammatory activity, the three *E. purpurea* extracts were fractionated by semi-preparative High performance liquid chromatography (HPLC), and its anti-inflammatory activity was also studied with LPS-stimulated primary macrophages. As expected, the fractions exhibited stronger reduction of inflammatory mediators (IL-6 and ROS/RNS), in comparison with the crude extract, through the drastic reduction of the phosphorylation of the p38 and ERK1/2 MAPK pathway. Moreover, in the experimental conditions used in this study, *E. purpurea* extracts showed more robust anti-inflammatory activity than two clinically prescribed anti-inflammatory drugs, namely celecoxib and dexamethasone. Therefore, *E. purpurea* extracts contain fractions with potent bioactivity for the treatment of inflammatory diseases by modulation of the p38 and ERK1/2 signaling pathways.

Acknowledgments: We acknowledge the financial support by the FCT to SFV (PD/BD/135246/2017), SMG (SFRH/BD/136814/2018), and CC (CEECIND/04058/2018), and the projects PATH (PD/00169/2013), Cells4_IDs (PTDC/BTM-SAL/28882/2017) and the NORTE 2020 Structured Project, cofunded by Norte2020 (NORTE-01-0145-FEDER-000021). We acknowledge the donors at the Hospital de Braga (Braga, Portugal) for providing buffy coats.

NOTES

Thank you for your participation!

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