Soft Materials with Dynamic Properties

17th ScSB Meeting

Helsingør, Denmark

April 23-26, 2024

TES2012

Welcome to Helsingør

Dear Biomaterials Scientists,

It is with great pleasure that we welcome you to attend the 17th ScSB Annual Meeting (ScSB2024) at in the charming city of Helsingør, Denmark. Helsingør is a town rich in history and culture, with notable attractions such as Hamlet's Castle by Shakespeare, a UNESCO world heritage site. This year's focus is on ***Soft Materials with Dynamic Properties***, and we believe that you will find this scientific gathering informative and engaging.

Our team has carefully curated an outstanding scientific program that explores the enormous potential of soft biomaterials and their applications in the medical and biomedical fields. We have invited distinguished researchers to share their expertise with the aim of exchanging ideas and fostering collaboration.

In addition to the scientific program, we have organized a series of social activities that will provide great opportunities for networking and interacting with our more than 130 esteemed Scandinavian and international scientists. You will have the opportunity to immerse yourself in the essence of the 'Danish Hygge' concept, emphasizing comfort and
coziness.

We are confident that ScSB2024 will be a memorable and rewarding experience. Thank you for attendance and we are delighted to welcoming you to Helsingør!

Best regards,
The ScSB2024 Organizing Committee

Organizers

Prof. Assoc. Alireza Dolatshahi-Pirouz
(Event Manager)

Dr. Tatiane Eufrasio-da-Silva
(Event Coordinator)

M.Sc. Sinziana Revesz
(Support)

Ms. Christel Pia Wagner
(Venue Manager)

Dr. Firoz Babu Kadumudi
(Support)

Dr. Morteza Alehosseini
(Support)

About ScSB

The Scandinavian Society for Biomaterials (ScSB) was founded as a non-profit organization in May 2008. The main focus of our activities are centered on organizing annual Biomaterials meetings in Scandinavian, Nordic and Baltic states, and thereby promoting cross-fertilization of Biomaterials research in these regions. In addition to academic researchers, ScSB regards students and industry as very important members of the society.

Our objectives:

•To encourage progress in the field of Biomaterials in all aspects, including research, teaching, and clinical applications;

•To promote the propagation of scientific information in the field of Biomaterials;

•To promote the interaction between the different disciplines of the field of Biomaterials as well as between basic research and applied practice;

•To co-operate with other scientific organizations, governmental and private

•To develop structured activities on issues involving Biomaterials;

•To stimulate the creation of research programs on Biomaterials primarily among member states and secondly on a wider international level.

To reach these goals, the following are promoted:

•The organization of an Annual Meeting at which members of the Society are encouraged to report on their research and development work;

•The organization and management of congresses, workshops, and advanced courses on special themes that have been suggested by the members;

•The promotion of young Biomaterials scientists active in member states at national and international levels;

•The setup of "ad hoc commissions" to address urgent problems in the area of Biomaterials.

Sponsors

We gratefully acknowledge all financial support from our generous sponsors: Optics11Life, Cellink, Ourobionics, Cybosense, Noubio, Greenelit, Ourobionics, TeamBioEngine and Department of Health Technology – DTU, we suggest you to visit their stands and websites to know more about their products.

TeamBioEngine

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DTU Health Tech
Department of Health Technology

DTU

Program

Tuesday, April 23rd

Check-in	15:00 - 17:25	Marienlyst Hotel & Conference check-in
Conference Opening	17:30 - 17:55	Welcome program and Opening of ScSB2024 meeting
Dinner	18:00 - 19:50	Welcome Dinner
19:55 – 21:15 SCIENTIFIC PROGRAMME A Chair: Nihal Engin Vrana	19:55 - 20:30	**Plenary Speaker** **Prof. Aldo Boccaccini, Uni-Erlangen-Nuremberg, Germany** *Exploring biologically active ions (bioinorganics) for applications in tissue engineering by investigating bioactive glasses and their composites*
	20:35 - 20:45	**J Locs, Riga Technical University, Latvia** *Synthesis and room temperature consolidation of octacalciumphosphate*
	20:47 - 20:59	**J T Koivisto, Karolinska Institutet, Sweden** *Biomaterial tools for studying the early phase of osteoblast differentiation and building a 3D in vitro bone model*
	21:01 - 21:13	**H Ben Amara, University of Gothenburg, Sweden** *From osteopromotion at the interface to adiposity accumulation in the peri-implant bone marrow: exploring bone response to biodegradable magnesium implants*
Poster & Social Event	21:15 - 22:15*	Poster & Social Event *It is voluntary to stay until 22:15.

Program

Wednesday, April 24th

Leisure & Breakfast** (** Spa access: 6:00 - 09:00 am)	06:30 - 07:00 07:00 - 08:30	Yoga Class Breakfast (At Isabel restaurant, it is provided until 10:00 am.)
08:35 – 10:05 SCIENTIFIC PROGRAMME B Chair: Manuel Mazo Vega	08:35 - 09:10	**Keynote Speaker 1** **Prof. Molly Stevens, Imperial College, UK** *Designing and translation biomaterials for advanced therapeutics and ultrasensitive biosensing*
	09:15 - 09:27	**L Hosta-Rigau, Technical University of Denmark, DK** *Multifunctional red blood cell substitutes: a novel approach toward safer and more versatile oxygen carriers*
	09:29 - 09:39	**G Bor, Technical University of Denmark, DK** *Metal phenolic network-integrated metal-organic framework-based oxygen carriers for enhanced blood circulation*
	09:41 - 09:51	**E Oreja, University of Oslo, Norway** *Tannic acid-protein interaction in coated titanium surfaces*
	09:53 - 10:03	**T Mitra, University of Galway, Ireland** *3D lung tumour hydrogel phantom: a simulation test bed for microwave*
Break	10:05 - 10:25	Coffee break
10:30 – 12:00 PARALLEL SCIENTIFIC SESSIONS (SEE NEXT PAGE)		

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Program

Wednesday, April 24th

	10:30 - 11:05	**Keynote Speaker 2** **Prof. Yu Shrike Zhang, Harvard Medical School, USA** *Light-based vat-polymerization bioprinting towards tissue fabrication*
10:30 – 12:00 **SCIENTIFIC PROGRAMME C1** Chair: Menglin Chen	11:10 - 11:20	**VS Parihar, Tampere University, Finland** *Two-step crosslinking approach for development of photo-crosslinkable biomaterial inks for extrusion – based 3D bioprinting*
	11:22 - 11:32	**A Pylostomou, Riga Technical University, Latvia** *Condensed orchestration of mesenchymal stromal cells via dynamic temperature-actuated hydrogels*
	11:34 - 11:46	**X Wang, Åbo Akademi University, Finland** *Xeno-free and photocurable nanocellulose-based macroporous-gel bioprinting in service of 3D cell culture*
	11:48 - 11:58	**R Bellan, Eindhoven University of Technology, The Netherlands** *Tuning the assembly of ureido-pyrimidinone molecules in living systems*
	11:15 - 11:19	**M Motadayen, Aarhus University, Denmark** *Research of biodegradable nanocomposite based on polyvinyl alcohol and tungsten nanoparticles*
	11:20 - 11:24	**K Hopia, Tampere University, Finland** *Novel adhesive and 3D-bioprintable bioinks for treatment of corneal tissue engineering*
	11:25 - 11:29	**S Yu, Karolinska Institutet, Sweden** *Versatile gallium-based nanoparticles as antibacterial agents*
11:15 – 11:50 **SCIENTIFIC PROGRAMME C2** **(Project in 3 minutes)** Chair: Fatemeh Ajalloueian	11:30 - 11:34	**R Jing, Alborg University, Denmark** *Supramolecular self-healing UV-curable hydrogels without photo-initiator using modified dextrans*
	11:35 - 11:39	**D Boscaro, Norwegian University of Science and Technology, Norway** *Alginate encapsulated bone spheroids: approaches to study bone cells in 3D*
	11:40 - 11:44	**Y Merhi, Århus University, Denmark** *Unlocking piezoelectric potential: terahertz spectroscopy characterization of crystallinity changes in flexible and bioresorbable poly-l-latic acid with tunable piezoelectric properties through mechanical stretching*
	11:45 - 11:49	**V Guerrero-Florez, Linköping University, Sweden** *Protease-active delivery of antimicrobial peptides from mesoporous silica*

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Program

Wednesday, April 24th

Lunch	12:05 - 13:10	Lunch
13:15 – 14:40 SCIENTIFIC PROGRAMME D Chair: Line Hagner Nielsen	13:15 - 13:50	**Keynote Speaker 3** **Prof. Gorka Orive, Univ. of Basque Country, Spain** *Development of the next generation 3D cyborganic scaffolds for tissue regeneration*
	13:55 - 14:05	**Sponsors presentations: Cellink**
	14:07 - 14:17	**Sponsors presentations: Optics11Life**
	14:19 - 14:29	**Sponsors presentations: Ourobionics**
	14:31 - 14:41	**Sponsors presentations: Cybosense**
Leisure	14:45 - 16:35	Social Events
Break	16:40 - 17:00	Coffee break
17:05 – 18:20 SCIENTIFIC PROGRAMME E Chair: Sabine van Rijt	17:05 - 17:40	**Keynote Speaker 4** **Prof. Tina Vermonden, Utrecht University, The Netherlands** *Injectable hydrogels for ocular drug delivery*
	17:45 - 17:55	**V Tsikourkitoudi, Karolinska Institutet, Sweden** *NAD-boosting nanoparticle drug delivery system for glaucoma treatment*
	17:57 - 18:07	**J Amirian, Riga Technical University, Latvia** *Novel injectable chitosan methacrylate (ChiMa) hydrogel reinforced with oxidized cellulose nanofibers methacrylate (OCNFMA) for hemostatic and wound healing agents*
	18:09 - 18:19	**E Zattarin, Linköping University, Sweden** *Multifunctional nanocellulose-based hybrid hydrogel wound dressing for wound infection treatment*
Break	18:20 - 18:39	Coffee Break

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Program

Wednesday, April 24th

18:40 – 19:50 SCIENTIFIC PROGRAMME F (Project in 3 minutes) Chair: Daniel Aili	18:40 - 19:15	**Keynote Speaker 5** **Prof. Rui Reis, University of Minho, Portugal** *Addressing complexity in tissue engineering and ways to engineer different complex in vitro models using polymeric hydrogels and stem cells*
	19:20 - 19:24	**H Gao, University of Groningen, The Netherlands** *Tailored design of macrophage-oriented metal-organic frameworks for robust gene silencing*
	19:25 - 19:29	**M Jolic, University of Gothenburg, Sweden** *Can mechanically overload implants re-osseointegrate?*
	19:30 - 19:34	**Q Wang, Åbo Akademi University, Finland** *Aqueous two-phase emulsion-based bioink enhanced by phosphorylated cellulose nanofibers*
	19:35 - 19:39	**R Shamasha, Linköping University, Sweden** *Biofabrication of granular hydrogel-based bioinks for dermal regeneration*
	19:40 - 19:44	**S Revesz, Technical University of Denmark, DK** *Bioactive, tough, and electroactive hydrogels for skeletal muscle tissue engineering*
	19:45 - 19:49	**JAV Cordova, Technical University of Denmark, DK** *Cardiac patch of a novel self-healing hydrogel for mending post-infarction defects*
Leisure & Dinner	20:00 - 22:00	Dinner & Social Event

Program

Thursday, April 25th

Leisure & Breakfast** (** Spa access: 6:00 - 09:00 am)	07:00 - 08:30	Breakfast (at Isabel restaurant, it is provided until 10:00)
08:35 – 09:55 **SCIENTIFIC PROGRAMME G** **Chair:** **Niels B. Larsen**	08:35 - 09:10	**Keynote Speaker 6** **Prof. Akhilesh Gaharwar, Texas University, USA** *Engineered biomaterials for reparative and regenerative medicine*
	09:15 - 09:27	**F Ajalloueian, Technical University of Denmark, DK** *Loading and coating of bacterial compounds into/onto electrospun nanofibers for medical applications*
	09:29 - 09:41	**S Naeimipour, Linköping University, Sweden** *Enzimatically triggered deprotection and cross-linking of thiolatedpolymer for 3D cell culture and bioprinting application*
	09:43 - 09:53	**A Solberg, RISE PFI/RISE Material and Surface Design, Norway/Sweden** *Biopolymers-based inks for 3D printing*
Break	09:55 - 10:15	Coffee break
10:20 – 11:55 **SCIENTIFIC PROGRAMME H1** (Parallel Session) **Chair:** **Hajar Maleki**	10:20 - 10:55	**Keynote Speaker 7** **Prof. Fergal O'Brien, RCSI, Ireland** *Biomaterials scaffolds for the delivery of gene therapeutics for enhanced tissue repair*
	11:00 - 11:12	**J U Lind, Technical University of Denmark, DK** *Multi-material printing of bio-hybrid systems*
	11:14 - 11:26	**H Maleki, University of Cologne, Germany** *Self-assembly driven biomimetic 3Dprinted hybrid aerogel-based theragenerative scaffolds for bone regenerative and bone cancer therapy*
	11:28 - 11:40	**C P Pennisi, Aalborg University, Denmark** *Hydrogel-elastomer interface engineering for cyclic mechanical stimulation of bioprinted 3D tissue constructs*
	11:42 - 11:52	**M Heilala, Aalto University, Finland** *Fibrin stiffness regulates phenotypic plasticity of metastic breast cancer cells*
11:05 – 11:45 **SCIENTIFIC PROGRAMME H2** (Parallel Session) **Chair:** **Masoud Hasany**	11:05 - 11:17	**V Sanjairaj, New York University Abu Dhabi, U. Arab Emirates** *Bioactive tissue scaffolds from decellularised ascidian tunic*
	11:19 - 11:31	**R V Ramachandran, Karolinska Institutet, Sweden** *Cost-effective antibacterial wound dressing comprising nanofibers infused with flame-made Ag/SiO2 nanoparticles*
	11:33 - 11:43	**N Reustle, Linköping University, Sweden** *3D breast cancer models to study proteolytic activity in vitro*

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Program

Thursday, April 25th

Break	11:55 - 12:55	Lunch
13:00 – 14:20 SCIENTIFIC PROGRAMME I Chair: Mani Diba	13:00 - 13:35	**Keynote Speaker 8** **Prof. Patricia Dankers, Eindhoven University, Netherlands** *Engineering supramolecular polymer hydrogels into synthetic extracellular matrices*
	13:40 - 13:52	**N Ferraz, Uppsala University, Sweden** *Investigating the effect of conjugation chemistry on the bioactivity of immobilized host defense peptides*
	14:06 - 14:16	**S Nikzad, Technical University of Denmark, DK** *Water-based inks for use in extrusion 3d printed instrumented tissues*
Leisure	14:20 - 16:20	Social Events
Break	16:25 - 16:45	Coffee break
16:50 – 18:40 SCIENTIFIC PROGRAMME J Chair: Johan Ulrik Lind	16:50 - 17:25	**Keynote Speaker 9** **Prof. Niels B Larsen, Technical University of Denmak, DK** *Soft materials and methods for engineering oxygen supply*
	17:30 - 17:42	**M Bjorninen, Tampere University, Finland** *Creating soft 3D tissue models for studying ischemia in body-on-a-chip systems – A research overview of the centre of excellence in body-on-a-chip- research*
	17:44 - 17:54	**J van Hoorick, BIO INX, Belgium** *ASTROCARDIA: A vascular heart-on-a-chip model in space to study cardiac ageing*
	17:56 - 18:08	**M Chen, Aarhus University, Denmark** *Exploring electrohydrodynamics based functional nanofibers as multi-dimensional nano-interfaces*
	18:10 - 18:40	**ScSB 2024 GA Meeting and Board**
Break	18:45 - 19:00	Coffee break

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Program

Thursday, April 25th

19:00 – 20:05 **SCIENTIFIC PROGRAMME K** **Chair:** **Winnie E Svendsen & Torben Moos**	19:00 - 19:35	**Keynote Speaker 10** **Prof. Sarah Heilshorn, Stanford University, USA** *Dynamic biomaterials to enable personalized tissue mimics*
	19:40 - 20:05	ScSB2024 Awards/Announcements & Pictures
Leisure & Dinner	20:10 - 22:00	Dinner & Conference closing

Friday, April 26th

Leisure & Departure** **(** Spa access: 6:00 - 09:00 am)**	07:00 - 10:00	Breakfast
	10:00 - 11:00	Hotel check out

Invited Speaker

Exploiting Biologically Active Ions (Bioinorganics) for Applications in Tissue Engineering by Investigating Bioactive Glasses and their Composites

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The development of ion doped bioactive glasses (BGs) and their composites for tissue engineering applications will be discussed comprehensively covering the state of the art in the field and presenting current results and future directions. It is well known that biochemical reactions occurring at the interface between BGs and the biological environment induce the release of BG ionic dissolution products, which affect cell behavior and provide opportunities for BG applications in both hard and soft tissue regeneration. Different BG compositions, including silicate, phosphate and borate BGs, can be designed with the capability to release biologically active ions which have positive effects on vascularization, wound healing, bone, muscle and nerve tissue repair. The immunomodulatory effects of BG ionic dissolution products are also being investigated. Given the importance of angiogenesis on tissue regeneration, the specific design of BG compositions (e.g. containing B, Co, Cu, Li, etc.) to achieve an angiogenic effect will be discussed based on the results of in-vitro studies, in particular showing the increase of the secretion of vascular endothelial growth factor (VEGF) from stem cells. In addition, technologies being investigated to fabricate flexible or malleable BG based scaffolds will be presented. These include: random and aligned fibers made from both pure BGs and BG-biopolymer composites, BG particulate incorporation in hydrogels for 3D (bio)printing and flexible polymeric scaffolds filled or coated with BG nanoparticles. In particular, recent results on composite hydrogels incorporating BG particles and therapeutic biomolecules for inducing synergistic effects (angiogenesis, antibacterial) will be presented. Such applications involve ion releasing BG nanoparticles incorporated in cell laden printable hydrogels. Challenges and opportunities in the field focusing on the effect of ionic dissolution products on encapsulated cells will be discussed.

Prof. Dr. Aldo R. Boccaccini,

Aldo R. Boccaccini is Prof. of Materials Science (Biomaterials) and Head of the Institute of Biomaterials at Uni of Erlangen-Nuremberg, Germany, and a visiting Prof. at Imperial College London, UK. He has an Engineering degree from Instituto Balseiro, Argentina (1987), and a Doctorate in Engineering Sciences (Dr.-Ing.) from RWTH Aachen University, Germany (1994). He had post-doctoral appointments at Uni of Birmingham, UK (1994-1996), and at the University of California, San Diego, USA (1996-1997). The research activities of Prof. Boccaccini are in the field of ceramics, glasses and composites for biomedical, functional and/or structural applications with focus on bioactive materials, scaffolds for tissue engineering, biofabrication and antibacterial coatings. He has been a visiting professor at different universities around the world and has given more than 150 presentations at international conferences. Boccaccini has published more than 1000 scientific papers, 25 book chapters and co-edited 8 books. His work has been cited more than 67,000 times (h-index = 112, Scopus®). He has received multiple awards and honors, including the Materials Prize of the German Materials Society (2015). Boccaccini is also an elected member of the World Academy of Ceramics, the National Academy of Engineering and Applied Sciences of Germany (acatech) and fellow of the European Academy of Sciences (EurAsc). He was conferred the degree of Honorary Doctor of Philosophy at Åbo Akademi University, Turku, Finland. Boccaccini currently serves as vice-president of the Federation of European Materials Societies (FEMS). He has been a member of the Council of the European Society for Biomaterials (ESB) since 2015, serving as ESB vice-president (2020-2023).

Designing and Translating Biomaterials for Advanced Therapeutics and Ultrasensitive Biosensing

University of Oxford, Kavli Institute for Nanoscience Discovery,
Department of Physiology, Anatomy and Genetics,
Department of Engineering Science
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In this talk I will discuss highlights of our nanomedicine portfolio including nanosensors for diagnosing and monitoring infectious and non-communicable diseases [Nature 2019; Nature Nanotechnology 2022], nanomimicking approaches for virus and parasite host cell entry inhibition recently demonstrated for malaria, HSV-2 and SARS-CoV-2 [ACS Central Science 2022], high molecular weight polymer carriers for enhanced delivery of saRNA therapeutics [ACS Nano 2020], and photo-responsive nanoreactors inspired in the circadian rhythms [Nature Chemistry 2023]. I will present advances in Raman spectroscopy for high-throughput label-free characterization of single nanoparticles (SPARTA™) that allow us to integrally analyse a broad range bio-nanomaterials without any modification [Nature Comm 2018, Advance Materials 2021]. SPARTA™ has become an integral tool for the design of nanotherapeutics, with recent examples including DOPC-containing lipid nanoparticles for nucleic acid delivery and dendrimersome-based systems for controlled delivery of anti-bacterial drugs, and for profiling extracellular vesicles (EVs) for detection of breast cancer through a minimally invasive liquid biopsy. Finally, I will discuss how we are establishing effective translational pipelines to drive our innovations to clinical application while actively engaging in efforts towards the democratisation of healthcare [Nature Materials 2022].

Prof. Dr. Molly Stevens

Prof Molly Stevens FREng FRS is John Black Professor of Bionanoscience at the University of Oxford and also holds part-time professorships at Imperial College London and the Karolinska Institute. She graduated with a First-Class Honours BPharm degree from Bath University in 1995 and a PhD from the University of Nottingham in 2001. After postdoctoral research in the Langer Lab at MIT, she joined Imperial College London in 2004 as a lecturer and was promoted to Professor in 2008 as one of the youngest Professors ever in the history of the institution. Molly's multidisciplinary research balances the investigation of fundamental science with the development of technology to address some of the major healthcare challenges. She is a serial entrepreneur and the founder of several companies in the diagnostics, advanced therapeutics and regenerative medicine space. Her work has been instrumental in elucidating the bio-material interfaces. She has created a broad portfolio of designer biomaterials for applications in disease diagnostics and regenerative medicine. Her substantial body of work influences research groups around the world (>430 publications, h-index 104, >44k citations, 2018, 2021 and 2022 Clarivate Analytics Highly Cited Researcher in Cross-Field research). Molly holds numerous leadership positions including Director of the UK Regenerative Medicine Platform "Smart Acellular Materials" Hub, Deputy Director of the EPSRC IRC in Early-Warning Sensing Systems for Infectious Diseases and Scientist Trustee of the National Gallery. She is Fellow of the Royal Society and the Royal Academy of Engineering (UK), Foreign Member of the National Academy of Engineering (USA) and International Honorary Member of the American Academy of Arts and Sciences.

Light-based Vat-Polymerization Bioprinting towards Tissue Fabrication

Department of Medicine, Harvard Medical School,
Division of Engineering in Medicine,
Brigham and Women's Hospital, Harvard Stem Cell Institute, Harvard University
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Three-dimensional (3D) bioprinting has emerged as a class of promising techniques in biomedical research for a wide range of related applications. Specifically, vat-polymerization techniques such as digital light processing (DLP), are highly effective methods of bioprinting, which can be used to produce high-resolution and architecturally sophisticated structures. Nevertheless, conventional DLP bioprinting systems are hampered by several key limitations such as their bulky footprints, their insufficient multi-material bioprinting capacities, and their usual requirements on mechanically strong materials for bioprinting of volumetric tissues due to the layer-by-layer fabrication mechanism. In this talk, I will discuss our recent efforts on developing various DLP-based platforms that successfully tackle these challenges. Additional discussions towards the more newly developed volumetric bioprinting strategy will also be covered. These platforms will likely provide new opportunities in constructing functional regenerative and tissue modeling products in the future.

Prof. Dr. Y. Shrike Zhang

Dr. Zhang is an Associate Professor in the Department of Medicine at Harvard Medical School and Associate Bioengineer in the Division of Engineering in Medicine at the Brigham and Women's Hospital. He is directing the Laboratory of Engineered Living Systems where the research is focused on innovating medical engineering technologies, including 3D bioprinting, organs-on-chips, microfluidics, and bioanalysis, to recreate functional tissues and their biomimetic models, for applications in regenerative medicine and personalized medicine. Dr. Zhang is an author of >300 peer-reviewed publications (>50 covers; citations >30,000, h-index = 87). His scientific contributions have been recognized by >45 awards.

A Scientific Journey: from Regenerative Medicine to Diagnosis and Health Sustainability

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The combination of smart materials, biological systems and bioelectronics is evoking a significant excitement and enthusiasm given its potential medical implications, especially in tissue regeneration and regenerative medicine. The present conference highlights this field of research, providing key examples of some of our latest 3D health-care implantable devices. The combination of cutting-edge cyborganic-integrated diagnostics and precision medicine may open new avenues in the healthcare sector of the future.

Prof. Dr. Gorka Orive

Gorka Orive, Full Professor of Pharmacy & Pharmaceutical Technology at the University of the Basque Country in Vitoria in Spain. Professor at the University UIRMI and scientist at Networking Biomedical Research Center on Bioengineering, Biomaterials and Nanomedicine, CIBER-BBN. He is one of the pharmacist (as scientists) with more international articles in Spain by the age of 46, with 370 international papers, 70 national articles, an H-index of 88 and over 27.000 citations. In the recent "Top Scientific Rankings" classification prepared by Stanford University, he heads the list of the most influential Spanish researchers in the world in Pharmacy and Pharmacology, being among the top 30 scientists in the world in this category, considering active researchers whose first article is in the 21st century. He has been also a relevant science communicator during the pandemic in twitter: @gorka_orive (more than 112,000 followers). He is scientific advisor of Pharma companies (GSK, Roche, Bayer, BTI,etc) and founded his own company Geroa Diagnostics (www.geroa.net) about Alzheimer´s diagnosis by salivary biomarkers.

Injectable Hydrogels for Ocular Drug Delivery

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In recent years, there has been considerable interest in the development of injectable hydrogels for drug delivery applications. These hydrogels offer controlled release of therapeutic agents at specific locations in the body. In order to render hydrogel materials injectable, their composition needs to be carefully designed to enable transitioning from a solution to a gel in response to certain stimuli such as a change in temperature of pH. This lecture will discuss the design of injectable thermosensitive hydrogels for the delivery of proteins and low molecular weight therapeutics aimed for local treatment of ocular diseases.

Prof. Dr. Tina Vermonden

Tina Vermonden obtained her Ph.D. degree in 2005 in Organic Chemistry at Wageningen University. In 2005, she joined the Department of Pharmaceutics at Utrecht University (The Netherlands) as a postdoc working on hydrogels as scaffolds for tissue engineering. During an exchange project, she worked on the diffusion of macromolecules in hydrogels at the Department of Pharmaceutics at the University of Minnesota (Minneapolis, USA) in the group of prof. R.A. Siegel in 2008. In 2009, she was appointed as assistant Professor and in 2020 as full Professor at the Department of Pharmaceutics in Utrecht. She participated as PI in several national and international collaboration programs and has been supervisor of 13 PhD students. In 2014, she was awarded the prestigious VIDI-grant and Aspasia-grant of Dutch Research Council for research on hydrogels that release drug-loaded micelles. In 2021, she obtained a VICI-grant for research on shrinking printing for kidney engineering. Since 2022, she acts as associate editor for the ACS journal Biomacromolecules.

Addressing Complexity in Tissue Engineering and Ways to Engineer Different Complex In Vitro Models Using Polymeric Hydrogels and Stem Cells

3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine; ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal.
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The selection of a proper material to be used as a scaffold, as a proper matrix, or as a bioink in 3D bioprinting approaches to support or encapsulate cells is both a critical and a difficult choice that will determine the success of failure of any tissue engineering and regenerative medicine (TERM) strategy. In our research group we have been mainly using natural origin polymers, including a wide range of marine origin materials, for many different approaches that allow for the regeneration of different tissues. Several innovative bioinks with quite specific properties were developed and proposed for several specific uses. We have also been optimizing the respective formulations for using these novel materials in distinct biomanufacturing strategies. Furthermore, an adequate cell source should be selected. In many cases efficient cell isolation, expansion and differentiation methodologies should be developed and optimized. We have been using different human cell sources namely: mesenchymal stem cells from bone marrow, mesenchymal stem cells from human adipose tissue, human cells from amniotic fluids and membranes and cells obtained from human umbilical cords. The potential of each biomaterials/cells combination and respective concentrations, as related to different manufacturing technologies, with details when appropriated focusing on bioprinting, to be used to develop novel useful regeneration therapies will be discussed. Several examples of TERM strategies to regenerate different types of tissues will be presented. The use of different cells and new ways to assess their interactions with different natural origin degradable scaffolds and bioinks will be described. A unique high-throughput platform to better understand material/cells interactions and optimise their performance and biological performance will be discussed. This rather innovative platform is based on the use of unique microfluidics-based approaches and allows for the engineering of novel complex in-vitro models, including disease tissue models.

Prof. Dr. Rui L. Reis

Rui L. Reis, PhD, DSc, Hon. Causa MD, Hon Causa PhD, FBSE, FTERM, member of NAE, FAIMBE, FEAMBES, is a Full Professor of Tissue Engineering, Regenerative Medicine, Biomaterials and Stem Cells at University of Minho (UMinho), Portugal. He is the Founding Director of the 3B's Research Group and Dean/President of the I3Bs – Institute for Biomaterials, Biodegradables and Biomimetics at UMinho. He is also the CEO of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine and has been the Global President of the Tissue Engineering and Regenerative Medicine International Soc. (TERMIS). He is Associate Editor of PNAS-NEXUS and is in the Editorial Board of several other relevant journals. He co-founded several companies that raised important private investments. His work has been cited more than 97000 times and he is listed in the annual Highly Cited Researchers 2022, list from Clarivate. He has been awarded many important international prizes.

Engineered Biomaterials for Reparative and Regenerative Medicine

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Engineered biomaterials have emerged as powerful tools for a range of biomedical applications, including regenerative medicine, drug delivery, bioprinting, and tissue modelling. These engineered biomaterials possess tunable biophysical properties, specific biochemical cues, and complex architecture, enabling precise control over cellular behavior. Our interdisciplinary lab, operating at the intersection of material science, bioengineering, cell biology, and genomics, has developed a range of biomaterial solutions to address challenges in the fields of reparative and regenerative medicine. In this talk, I will elucidate three pioneering approaches based on engineered biomaterials that our lab has developed for biomedical applications. First, I will discuss how we utilize engineered biomaterials to shape the transcriptomic and epigenetic landscapes of endogenous cells, steering them toward tissue repair and regeneration. This work has culminated in the creation of new class of biomaterials for stimulating bone and cartilage regeneration, as well as enhancing mitochondrial biogenesis and cellular energetics. Secondly, I will delve into our tailor-made nanomaterials aimed at the sustained and controlled release of therapeutic agents. Specifically, these nanomaterials target conditions such as osteoarthritis, angiogenesis, and wound healing. Our lab has developed a portfolio of nano-toolkits proficient in delivering a spectrum of bioactive molecules, ranging from small molecular drugs to large proteins, thereby offering versatile solutions for cellular programming. Lastly, I will showcase our efforts in 3D-bioprinting anatomical-size tissue constructs for both regenerative applications and tissue modeling. These advanced structures provide physiologically faithful tissue models capable of mimicking complex disease states, such as vascular pathophysiology and intricate vascularized tumor microenvironments

Prof. Dr. Akhilesh K Gaharwar

Dr. Akhilesh K. Gaharwar is the Presidential Impact Fellow and Professor in the Department of Biomedical Engineering at Texas A&M University. The goal of his lab is to design new biomaterials for reparative and regenerative medicine. His lab is leveraging principles from materials science, bioengineering, cell biology, and genomics to design smart and responsive biomaterials, with wide-ranging applications in the field of bioengineering. His research program is supported by the NIH, NSF, and DoD. Dr. Gaharwar has co-authored 140 journal articles and has an H-index of 69. His research accomplishments are recognized by more than 20 national and international awards, including the NIH New Innovator Award (DP2), Biomaterials Young Investigator Award, and Dean of Engineering Excellence Award. He is an elected fellow of the Biomedical Engineering Society (BMES), American Institute for Medical and Biological Engineering (AIMBE) and senior member of National Academy of Inventor (NAI). He currently serves as an Associate Editor of ACS Applied Materials and Interfaces.

Biomaterial Scaffolds for the Delivery of Gene Therapeutics for Enhanced Tissue Repair

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The COVID-19 pandemic has shown how revolutionary treatments based on gene modification technology has helped overcome a once-in-a-century pandemic and has given new momentum to gene therapy research for a myriad of applications. The field of regenerative medicine is well placed to be a beneficiary whereby, for example, gene therapy might be a valuable tool to avoid the limitations of local delivery of growth factors. While non-viral vectors (NVV) are typically inefficient at transfecting cells, our group have had significant success in this area using a scaffold-mediated NVV gene therapy approach for regenerative applications These gene activated scaffold platforms not only act as a template for cell infiltration and tissue formation, but also can be engineered to direct autologous host cells to take up specific genes and then produce therapeutic proteins in a sustained but eventually transient fashion. Similarly, we have demonstrated how scaffold-mediated delivery of siRNA and miRNA can be used to silence specific genes associated with reduced repair or pathological states. This presentation will provide an overview of ongoing research in our lab in this area with a particular focus on gene-activated biomaterials for promoting bone, cartilage, nerve and wound repair. Focus will also be placed on advances we are making in using 3D printing of gene activated bioinks to produce next generation medical devices for tissue repair. Acknowledgements: European Research Council Advanced Grant, ReCaP (agreement n° 788753).

Prof. Dr. Fergal J. O'Brien

Fergal O'Brien is Deputy Vice Chancellor for Research & Innovation, Professor of Bioengineering & Regenerative Medicine and Head of the Tissue Engineering Research Group in RCSI. He is a leading innovator in the development of advanced biomaterials for tissue repair with target applications in bone, cartilage, cardiovascular, ocular, respiratory and neural tissues. A major focus of ongoing research is to functionalise these scaffolds for use as delivery systems for biomolecules with a particular interest in the delivery of gene therapeutics to promote enhanced tissue repair. Since his faculty appointment, he has published over 300 journal articles and supervised over 50 doctoral students to completion and translated a number of regenerative technologies for bone and cartilage repair to the clinic through spin-out formation and licensing to industry. He is a recipient of numerous prestigious awards including a €3million Advanced Grant from the European Research Council, Membership of the Royal Irish Academy and the 2023 Marshall R. Urist, MD Award from the Orthopaedic Research Society for his sustained contribution to musculoskeletal research

Engineering Supramolecular Polymer Hydrogels into Synthetic Extracellular Matrices

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The extracellular matrix (ECM) is a complex, hierarchical assembly of various molecules held together via both covalent and noncovalent interactions. In order to make materials with comparable properties it is proposed that supramolecular hydrogels based on hydrogen bonding units are eminently suitable. An important challenge in the synthesis and formulation of a synthetic ECM, is besides the balance between dynamics and robustness, the introduction of complexity. This complexity might originate from hierarchical structures formed by assembly of our supramolecular monomers, or from bioactive molecules co-assembled with these monomers. Both parameters will heavily influence the function of the materials when brought into contact with cells. The bioactive function in our supramolecular hydrogels is based on small synthetic peptides, large ECM proteins, or carbohydrates. Here we show how to engineer these supramolecular polymer hydrogels into synthetic ECM matrices for the culture of cells and organoids. Importantly, we fundamentally study the effect of dynamic ligand presentation to cells and its role in controlling cell behavior.

Patricia Y. W. Dankers
Eindhoven Uni. of Technology, Inst. for Complex Molecular Systems, and Dep. of Biomedical Eng., Lab. of Chemical Biology, Eindhoven, The Netherlands

Patricia Dankers is professor in Biomedical Materials & Chemistry at the Eindhoven University of Technology (TU/e). She studied chemistry in Nijmegen, The Netherlands. Her PhD studies were performed at TU/e in the group of prof. E.W. (Bert) Meijer, on supramolecular biomaterials (2006). She worked for SupraPolix, and the University Medical Center, Groningen. Her second PhD thesis work was performed in medical sciences on kidney regenerative medicine, in Groningen (2013). She worked at Northwestern University, Chicago, USA (2010). She climbed every step on the academic ladder, starting in 2008, ending in 2017 as full professor. She received Veni, Vidi & Vici (2008, 2017, 2023) and ERC starting & ERC PoC (2012, 2017) grants. She has been awarded the KNCV Gold Medal (2020) and the Ammodo Award for Fundamental Science (2021). She is a co-founder of the spin-off companies UPyTher (2020) and VivArt-X (2022). She is one of the founders of the Research Center for Interactive Polymer Materials (IPM) in Eindhoven, funded by a Gravitation Grant (2022).

Soft Materials and Methods for Engineering Oxygen Supply

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Inadequate oxygen supply is currently the major factor limiting the engineering of tissues thicker than a millimeter, and numerous concepts to overcome this limitation have been proposed. We and others have approached the reproduction of vasculature function by microengineering massively parallel microfluidic 3D networks in materials open to oxygen- and nutrient-diffusion [Lab Chip 2017]. Liver tissue models are of great interest for drug development and disease modeling. They also pose a special challenge given the fragility and high oxygen consumption rate of hepatocytes as well as the need for a gradient in oxygen tension along each liver sinusoid, thought to be key in establishing the metabolically essential liver zonation. In the engineered microfluidic network, the liver-like 3D tissue is cultured between the perfusion channels, thereby shielding the sensitive hepatocytes from shear stresses of the medium flow. The developed platform has been employed for the culture of primary human hepatocytes at in vivo-like cell densities for weeks as well as culture of human induced pluripotent stem cell-based liver-like cell tissues for months [Acta Biomater 2023]. Gradients in oxygen tensions naturally develop within the tissue model due to cellular oxygen consumption, which cannot be predicted by numerical modeling without access to a ground truth. We have met this generic challenge by the development of a method for mapping the oxygen concentration in 3D within tissues using advanced confocal microscopy of embedded oxygen-sensitive microbeads [Lab Chip 2022, Adv Sci 2022].

Prof. Niels B. Larsen

Niels B. Larsen received his PhD in surface chemistry from University of Copenhagen in 1997, and has been a full professor since 2003, first at Riso National Laboratory in Denmark and since 2007 at Technical University of Denmark. Dr. Larsen's research has focused on development of advanced manufacturing methods and materials to engineer 2D and 3D static and dynamic environments at the micro- and nanometer scale to monitor and guide living systems, with key attention to scalability and automation of the developed technologies to industrial and clinical application. In recognition of his contributions to basic and applied materials and manufacturing research, Dr. Larsen received the Ellen and Hans Hermer's Prize in 2011 and the ATV Elastyren Prize in 2016. His current research interests focus on technologies to recapitulate the function of the human vascular network for tissue engineering applications. The technologies mainly target advanced in vitro 3D tissue culture but also address the requirements for extracorporeal and implantable devices. The key technology platform is high-resolution stereolithographic 3D printing in diffusion-open materials, for which Dr. Larsen has developed custom-build multi-color stereolithography systems and matching printing resins. This combination enables spatially selective solidification of different resin components to produce monolithic devices with locally widely varying diffusive and mechanical properties.

Dynamic Biomaterials to Enable Personalized Tissue Mimics

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Each individual is unique, yet pharmaceutical companies design the same therapies for all of us using lab mice. In the future, the biofabrication of personalized tissue mimics offers the exciting possibility of individualized therapies. However, current biofabrication methods are greatly hampered by a lack of dynamic materials that are simultaneously biofunctional and reproducible. A cell's behavior is directly influenced by its surrounding microenvironment; thus, ideally each cell type would be cultured in its own customizable biomaterial. To fulfill this need, my lab designs bespoke biomaterials that can be tailored to fit a range of applications. In one demonstration, I present a family of dynamic biomaterials that support the growth of patient-derived organoids, i.e. three-dimensional cell aggregates that demonstrate emergent, tissue-like behavior. While organoid cultures have the potential to revolutionize our understanding of human biology, current protocols rely on the use of complex, heterogeneous materials with large batch-to-batch variations. In contrast, our double-network hydrogels are formulated with recombinant biopolymers that are fine-tuned to display a reproducible range of biochemical and biomechanical properties. In a second example, I present a new "pick-and-place" 3D bioprinting strategy for the spatial positioning of organoids within a dynamic support matrix. Using this method, we can fabricate large tissue structures composed of multiple organoids that fuse together. We demonstrate potential applications in the fabrication of neural "assembloids" composed of dorsal- and ventral-patterned neural organoids together with patient-derived brain cancer spheroids. We envision that these two technologies will be used together in the future to create personalized tissue models of individual patients.

Prof. Dr. Sarah C. Heilshorn

Sarah Heilshorn is Professor and Associate Chair in the Materials Science & Engineering Department at Stanford University, where she also serves as Director of the Geballe Laboratory for Advanced Materials. Her laboratory integrates concepts from materials science and protein engineering to design bioinspired materials for regenerative medicine, organoid culture, and bioprinting. She is a fervent supporter of diversifying the research community. She is a Fellow of the American Institute for Medical and Biological Engineering and the Royal Society of Chemistry. She serves as Associate Editor of the journal Science Advances, Editor of Acta Biomaterialia, and on the Board of Directors for the TERMIS (Tissue Engineering & Regenerative Medicine International Society).

Oral Presentation

Synthesis and room temperature consolidation of octacalcium phosphate

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INTRODUCTION: Calcium phosphate (CaP)-based bone substitute materials have been at the forefront of bone grafting and regeneration materials. One of the interesting but at the same time challenging CaP phase to work with is octacalcium phosphate ($Ca_8(HPO_4)_2(PO_4)_4.5H_2O$; OCP). Being a precursor of the bone mineral, it exhibits a similar structure – apatite layers parallel to the (100) plane, with interlaying hydrated layers composed of an alterable number of water molecules. Many efforts have been placed into deciphering the OCP formation. Still, several background processes, influenced by supersaturation, molarity, pH, temperature, and mixing order/rate, have made it much harder. Furthermore, even though the scientific community has witnessed great progress in utilising and processing OCP in different systems, the core characteristics of the unique OCP crystal structure prevent it from undergoing high-temperature treatments.

METHODS: OCPs were synthesised via hydrolysis of α-tricalcium phosphate (α-TCP) and the co-precipitation route. The hydrolysis route was scaled up a hundredfold (100 mg→10 g). Phase transformation kinetics were tested by taking the samples at multiple time points between 1st 180th hour of the synthesis [1].

As an alternative OCP synthesis method, a novel, reproducible and ultra-fast co-precipitation synthesis process was developed and optimised to obtain tailor-made OCP.

OCP was densified at room temperature by applying a uniaxial pressure of up to 1500 MPa (modified cold sintering process).

For all steps within processes, phase and chemical compositions were analysed by using X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FT-IR) and Raman spectroscopy. SSA of the powders was determined by using the Brunnauer–Emmet–Teller method (BET), while the morphology of powders was investigated by a scanning electron microscope (SEM).

RESULTS: In the hydrolysis route, the gradual transformation from α-TCP to the OCP phase transpired through brushite (DCPD) as an intermediary phase. The increase in batch size increases the time necessary for complete hydrolysis. By varying the synthesis parameters in the co-precipitation method, it was possible to obtain OCP with a specific surface area (SSA) ranging from 20 to 90 m^2/g. Applying uniaxial high pressure to the OCP leads to significant densification of the ceramic, reaching more than 90% of theoretical density. The analysis of consolidated samples confirmed that the densification occurs without altering the phase composition of OCP.

DISCUSSION & CONCLUSIONS:

It was possible to upscale the synthesis of OCP via hydrolysis of α-TCP up to 10 g/batch, but the total synthesis time takes up to one week. The more rapid co-precipitation method leads to the formation of OCP even in just 15 minutes, but the yield is low, and thus, the process needs to be further developed and upscaled. As OCP is a metastable phase that transforms spontaneously and irreversibly to apatite (>80ºC), it is impossible to sinter it using conventional high-temperature sintering techniques. Thus, room-temperature high-pressure consolidation (cold sintering) is an appropriate technology to apply.

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Biomaterial tools for studying the early phase of osteoblast differentiation and building a 3D *in vitro* bone model

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INTRODUCTION: Developing *in vitro* models of bone remodeling requires a suitable 3D culture substrate to properly mimic the microenvironment in bone. For this aim, we produced composite scaffolds by combining 3D printed solid material (beta-tricalcium phosphate (β-TCP) or polyethylene glycol diacrylate (PEGDA)) and filling the compartmentalized structure with collagen-based hydrogel and cells. We also developed mesoporous silica nanoparticles (MSNs) loaded with fluorescent dyes to enable easy cell labeling and tracking.

METHODS: The β-TCP was direct ink 3D printed from ternary capillary suspension and PEGDA was extrusion 3D printed and used as non-bioactive control. TeloCollagen® (3 & 6 mg/ml) and Geltrex® (10 mg/ml) were used as the soft, organic component. Mouse origin MC3T3-E1 pre-osteoblasts or MLOY-4 osteocytes were cultured for maximum 14 days ($5*10^6$ cell/ml). changes in hydrogel micro- and macromechanical properties were measured using Micro Particle Tracking based microrheology (Ø 0.2 µm green fluorescent polystyrene tracers embedded in hydrogel during gelation), bulk rotational rheometry, and uniaxial compression on days 0, 2, 4, 7, 14. MSNs were surface modified with polyethyleneimine (PEI) and further derivatized with acetic anhydride (ACA), succinic anhydride (SUC), and polyethylene glycol (PEG) to evaluate the effect of surface charge on nanoparticle uptake individually and as co-culture for selective uptake. Cytotoxicity of MSNs was studied with CellTiter-Glo® assay and migration of cells were assessed for 62 hours.

RESULTS: Rapid remodelling of hydrogel matrix was seen from day 4 onwards in the 3 mg/ml TeloCollagen® containing MC3T3-E1 samples, first as matrix degradation and then increase of stiffness. The stiffer TeloCollagen® had less cell activity, both in migration and changes in mechanical properties. Microrheology showed tracer particles either elastically trapped or freely moving in viscous liquid (pores) of the hydrogel. An increase of the hydrogel porosity was detected on day 4. Without cells, all the hydrogels retained their structure and mechanical properties over 14 days.

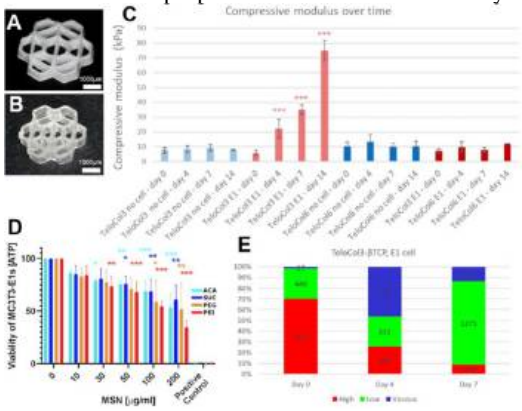

Fig. 1: 3D printed (A) β-TCP and (B) PEGDA scaffolds, (C) drastic increase in compressive modulus over time caused by cells, (D) cytotoxicity test for functionalized MSNs and their safe concentrations, (E) microrheological characterization of the hydrogel with the most remodeling activity shows appearance of microporosity (viscous), which then gets filled with cell-produced ECM (low stiffness elastic network).

CONCLUSIONS: The biomaterial tools developed in this project help to study the osteoblasts and the process of bone remodelling. The TeloCollagen® 3 mg/ml inside β-TCP scaffold is recognized as a promising 3D scaffold for bone *in vitro* studies. Additionally, the microrheology is shown to be a powerful tool to study changes in microstructural and micro-mechanical properties at cell-relevant scales throughout the culture period.

ACKNOWLEDGEMENTS: We wish to thank Dr. Kai Weißenbruch and Prof. Dr. Martin Bastmeyer, Cell- and Neurobiology, Karlsruhe Institute of Technology, for access to cell culture facilities. We acknowledge the funding from Tampere Institute for Advanced Studies, Oskar Huttunen Foundation, and Erik & Edith Fernström Foundation.

From Osteopromotion at the Interface to Adiposity Accumulation in the Peri-Implant Bone Marrow: Exploring Bone Response to Biodegradable Magnesium Implants

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INTRODUCTION: Metallic implants made of magnesium (Mg), which combine unique degradability and mechanical characteristics, are emerging as promising alternatives to permanent metallic implants in orthopaedic applications. Mg implants not only support osteosynthesis but are also known for their osteogenic properties [1]. This osteopromotive effect is often linked to the attenuation of inflammation. Yet, this view is challenged by the clinical and preclinical evidence [2] suggesting that initial inflammation is transiently amplified. The hypothesis of the present study was that, in comparison to nondegradable titanium implants, the degradation of Mg implants alters the cellular response and structural characteristics of bone at the interface with the implants and in the peri-implant marrow.

METHODS: Screws made of highly pure Mg (*Mg*; 99.998%), and commercially pure titanium (*Ti*; grade 4) were implanted in the proximal metaphyses of rats. After 3 and 28 d, implants and associated bone were retrieved. Quantitative polymerase reaction (qPCR) and immunohistochemistry enabled analyses of gene and protein expression of cells at the interface bone–implant and in the surrounding bone marrow. Histomorphometry and compositional studies via energy dispersive x-ray spectroscopy and micro-Raman spectroscopy of the newly deposited bone and the Mg degradation layer at the implant surfaces (n = 5-7/group/timepoint) were performed. Kruskal–Wallis and Mann–Whitney tests were used for statistics ($p<0.05$).

RESULTS: Compared to Ti implants, cells at the interface between bone and Mg exhibited a transient upregulation of genes related to inflammatory cytokines, proinflammatory macrophage, osteoclastogenesis, and neoangiogenesis. At 28 d, Mg implants featured a superior bone–implant contact (***Fig. 1a***), with a degradation layer rich in calcium and phosphorus at their surface. The interfacing bone displayed a higher Ca/P ratio and more Mg, with younger bone features indicated by Raman spectroscopy. Furthermore, in line with the upregulation of adipogenesis genes, increased density and size of adipocytes were noted in the bone marrow with a persistently higher density of CD68-immunopositive inflammatory cells surrounding Mg implants (***Fig. 1b***).

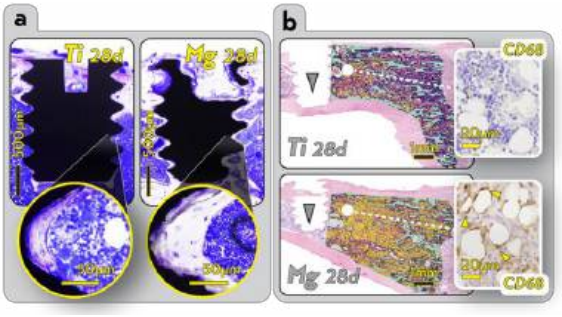

Fig.1: a) 28 d-histology showing enhanced bone formation at Mg implant surfaces compared to Ti; b) Increased bone marrow adiposity (yellow; Marrowquant [3]) near Mg implants (grey arrowhead), with right insets highlighting CD68-immunoreactive cells (yellow arrowheads).

DISCUSSION & CONCLUSIONS: Mg implants initially elicit a transient proinflammatory milieu that reinforces reparative osteogenesis at their surface. However, their degradation also causes compositional alteration in the interfacial bone with a previously unknown proadipogenic response and a persistent low-grade inflammation in the peri-implant bone marrow. This suggests the need for rigorous tailoring of Mg implants, and a vigilant monitoring of the adjacent bone marrow.

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Multifunctional Red Blood Cell Substitutes: A Novel Approach Toward Safer and More Versatile Oxygen Carriers

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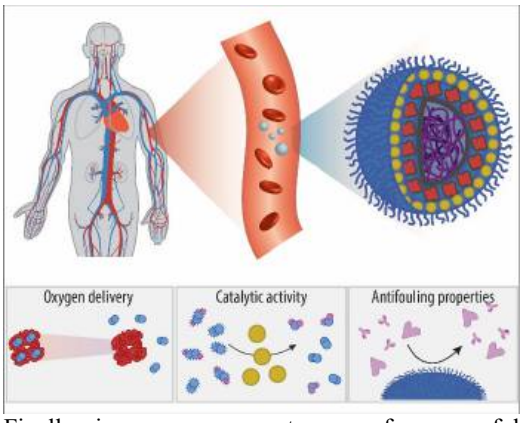
INTRODUCTION: The utilization of donor red blood cells (RBCs) in blood transfusions has been a crucial life-saving procedure, particularly for patients experiencing substantial blood loss. However, intricate processes such as pathogen testing and donor-recipient cross-matching, coupled with the limited shelf life of donor RBCs, pose significant challenges, leading to critical delays in emergency situations. The absence of stockpiles for acute disasters further underscores the need for alternative solutions, making blood substitutes highly sought after.

One prominent class of blood substitutes is hemoglobin (Hb)-based oxygen carriers (HBOCs), leveraging Hb as the primary component of RBCs responsible for oxygen transport.[1] Despite intense efforts, no HBOC has gained clinical approval in the USA or Europe. This fact can be attributed to the initial too rapid development of blood substitutes in the 1980s, due to fear of HIV contaminated blood, which resulted in several failures mostly due to unacceptable toxicities.

Significantly, although research endeavors have primarily concentrated on the creation of straightforward oxygen carriers, it is crucial to recognize that beyond oxygen delivery, biological RBCs play vital roles in carbon dioxide transport, nitric oxide (NO) production, and antioxidant functions — all of which are essential and life-saving functions. Thus, this presentation introduces the latest research on multifunctional RBC substitutes, moving beyond the exclusive focus on oxygen delivery to an integrated, flexible platform capable of performing multiple life-saving functions.

RESULTS: Unlike biological RBCs, which prevent metHb conversion through antioxidant enzymes, our substitutes utilize antioxidant nanozymes (NZs), specifically cerium oxide nanoparticles (CeO_2NPs), gold NPs (AuNPs), and Au nanoclusters. These NZs demonstrate catalytic depletion of reactive oxygen species and minimize Hb oxidation into metHb.

Biological RBCs possess the capability to release NO and induce NO production from the endothelium through ATP release. Consequently, native RBCs facilitate NO-dependent vasorelaxation, leading to the dilation of blood vessels as blood transitions from arteries to veins, ultimately improving oxygen delivery. The first generation of HBOCs resulted in elevated mortality rates, primarily attributed to NO scavenging. Given that NO serves as a crucial vasodilator, its removal by HBOCs leads to vasoconstriction and subsequent cardiovascular complications. We propose that by integrating NO-releasing subunits, thereby mimicking a key function of biological RBCs, we can potentially overcome the toxicity issues associated with the first HBOCs generation. Thus, we have investigated the potential of several NZs (i.e., CeO_2NPs, AuNPs and platinum NPs) to trigger NO-release from endogenous and synthetic NO-donors.



Finally, since an ever-present concern for successful RBCs substitutes is to achieve long circulation in the bloodstream, we have developed stealth coatings which include PEG but also decoration with membranes extracted from native RBCs, which are able to remain in circulation for up to 120 days.

This research represents a significant step towards developing safer and more versatile RBC substitutes, addressing critical issues associated with traditional blood transfusions and paving the way for innovative solutions in emergency medical interventions.

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Metal phenolic network-integrated metal-organic framework-based oxygen carriers for enhanced blood circulation

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INTRODUCTION: Due to limitations in blood transfusion, including availability, storage requirements, shelf life, and blood type compatibility, hemoglobin-based oxygen carriers (HBOCs) have emerged as a promising strategy for creating universal red blood cell (RBC) substitutes, particularly in prehospital care [1]. Recently, metal−organic framework-based particles (MOFs), have gained significant attention for encapsulation of hemoglobin (Hb) due to their high encapsulation efficiency and competitive biocompatibility [2,3]. Despite their attractiveness in developing HBOCs, the relatively low structural stability of most MOFs in biologically relevant fluids (e.g., blood serum/plasma or cell media) has limited their *in vivo* applications [4]. Herein, we incorporate metal-phenolic networks (MPN) into Hb-loaded MOF-based nanoparticles (Hb@MOF/MPN-NPs) to enhance their stability in biologically relevant fluids. Given that surface modification of NPs with polyethylene glycol (PEG) is a widely used strategy to prolong circulation time, we further decorate Hb@MOF/MPN-NPs with PEG. Next, we compare the *in vivo* behaviour of non-PEGylated and PEGylated Hb@MOF/MPN-NPs by evaluating their pharmacokinetic properties and biodistribution after intravenous injection (I.V.) in healthy mice.

METHODS: Prior to *in vivo* studies, we followed the effect of mouse plasma on stability of produced NPs and evaluated the changes in their size distribution, zeta potential and Hb release profile. The blood compatibility of produced NPs, including coagulation and complement activation was also investigated. After I.V. injection into the mice, the biodistribution of fluorescently labelled NPs was further studied by using micro-computed tomography (μCT) (**Fig.1**). Lastly, the pharmacokinetics properties were determined using a non-compartment model.

RESULTS: The biodistribution results showed the majority of injected non-PEGylated and PEGylated NPs were primarily localized in the liver and kidneys, which is in line with free Hb.

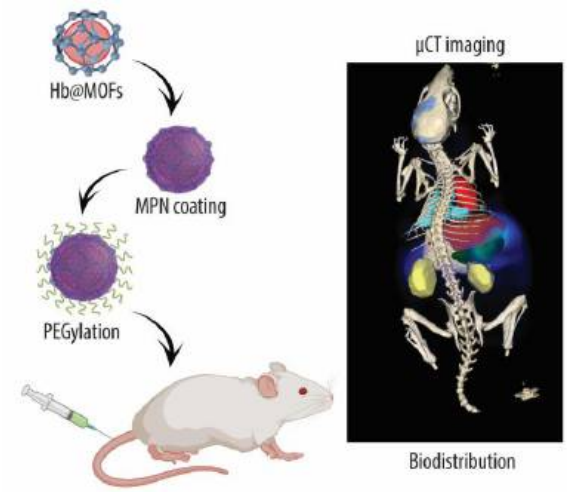

Fig. 1: Schematic overview of the study.

Pharmacokinetic studies further revealed that both PEGylated and non-PEGylated Hb@MOF/MPN-NPs provided prolonged blood elimination half-life ($t_{1/2\beta}$), compared to free Hb. However, the advantage of PEGylation over MPN-coating alone was not observed in extending blood circulation time.

DISCUSSION & CONCLUSIONS: Our findings provide an understanding of *in vivo* behaviour of non-PEGylated and PEGylated Hb@MOF/MPN-NPs may have implications for the future design of long-circulating HBOCs.

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Tannic acid-protein interaction in coated titanium surfaces

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INTRODUCTION: Interaction between proteins and surfaces is a determining factor that affects the biomaterial integration. Proteins adsorption is the first step that occurs when a foreign body is in contact with physiological fluids. Therefore, understanding the conformation, surface coverage and interactions is key to predict how body and cells will interact with the adsorbed proteins in the biomaterial surface [1]. Our aim is to understand how proteins present in blood interact with tannic acid (TA) coated titanium (Ti) surfaces.

METHODS: Protein-TA interaction was studied by different methods. Real time binding properties were measured at two different pH, 6.8 and 7. 8 by using a quartz crystal microbalance with dissipation (QCM-D). Fourier-transform infrared spectroscopy (FTIR) and ultraviolet-visible spectroscopy (UV-VIS) were used to assess the chemical/physical interactions between TA and proteins present in blood proteins. FTIR measurements were performed by mixing TA and different protein solutions. The precipitate obtained was purified before the measurement. The UV-VIS spectrum of TA and proteins was acquired both in solution and deposited onto quartz slides. TA release and antioxidant capacity of TA coatings deposited on Ti discs were assessed following protein adsorption using the Prussian blue assay and the ABTS assay, respectively.

RESULTS: Fast protein adsorption on both bare Ti and TA coated sensors was observed with QCM-D (Fig. 1). While no difference was observed in adsorption kinetics between the surfaces, different protein concentrations led to differences in adsorbed layer thickness.

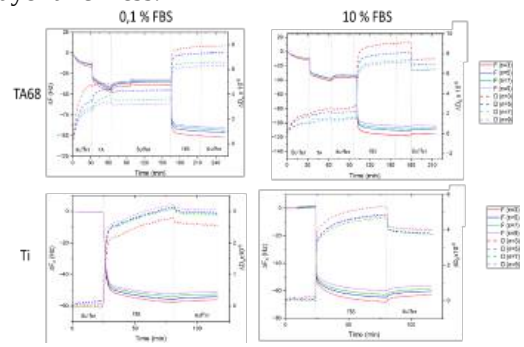

Fig. 1: Fig. 1: TA-protein deposition on QCM-D

Characteristic bands in the FTIR spectrum of TA were identified. Protein addition resulted only in peak shifts observed in FTIR, indicating a non-chemical interaction. Two characteristic bands for $\pi \rightarrow \pi^*$ transition of TA molecule were observed by UV-VIS. The intensity of one of the bands decreased in presence of proteins, indicating changes in the molecular environment.

The release studies showed that protein corona reduces TA release depending on the thickness of TA coating. The amount of TA released is higher when TA layer is thicker. However, the antioxidant capacity was not affected by the proteins corona regardless of the concentration of proteins or the TA coating thickness.

DISCUSSION & CONCLUSIONS: QCM-D results indicate that the thickness of the protein layer increased when protein concentration was higher because more proteins are available to bind on to the surface. The protein corona was thicker on TA-coated sensors compared to Ti sensors, irrespective of the pH at which TA coating was deposited. This can be attributed to the presence of increased number of OH groups involved in hydrogen bond interactions on TA coated surfaces [2]. No clear evidence of chemical interactions on the TA surface following protein adsorption was observed with either FTIR or UV-VIS spectroscopy, which further supports hydrogen bond formation between TA and the adsorbed proteins. While the adsorbed protein corona reduced TA release from the surface, the antioxidant capacity of the TA coating remains unaffected.

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3D lung tumour hydrogel phantom: a simulation test bed for microwave ablation

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INTRODUCTION:

Lung cancer is the most common cause of global cancer incidence and mortality, accounting for an estimated 2 million diagnoses and 1.8 million deaths [1]. In the European Union, it is the third most prevalent cancer with 388,000 annual cases. Despite advances in surgery, chemotherapy, and radiotherapy, the 5-year survival rate remains as low as 18% [2].

Thermal ablations have been shown to be effective therapies for minimally invasive treatment of tumours. In this process, the tumour is irreversibly damaged by the local application of extreme temperatures and eventual apoptosis of the tumour. It is mainly used to treat small tumours in patients who are not suitable for surgery [3]. The procedure is very similar to the surgical process in which the tumour is removed with a 5-10 mm margin of normal tissue [4]. To date, clinicians do not have meaningful data sets for real-time monitoring of lung tumour ablation.

In the present study, we developed a clinically relevant size tumour phantom with hyaluronic acid-tyramine hydrogel for real-time monitoring of the ablation process.

METHODS: The phantom model consisted of two parts: a normal tissue mimicking phantom and a tumour mimicking phantom. A microwave generator was used to irradiate the cell-loaded tumour phantom using 45 Watts of power for a treatment time of 7 minutes. Fibre optic cables were used as sensors to monitor temperature changes throughout the tumour phantom. Cancer cell viability was subsequently analysed by LIVE/DEAD staining using confocal microscopy.

RESULTS: During microwave ablation, the temperature in the tumour phantom was measured in real time at different positions and the average maximum temperature of 83, 53 and 45°C was recorded at 5, 10 and 15 mm. After ablation, assessment of A549 cell viability in the 3D lung tumour model showed a direct correlation between the maximum temperatures reached during the procedure and cell death (Fig. 1).

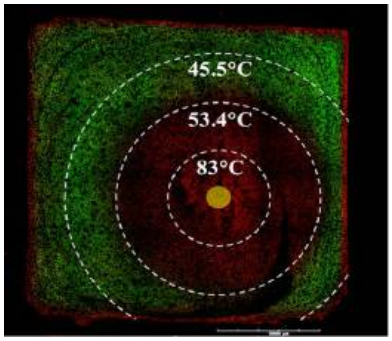

Fig. 1: Post ablation Live/dead image of A549s encapsulated in hydrogel slice. Dimensions of slice = 3cm x 3cm x 2mm. (temperatures at 5, 10 & 15 mm)

DISCUSSION & CONCLUSIONS: Fibre-optic monitoring demonstrated temperature differences throughout the tumour phantom. Approximately 83°C, 53°C, and 45°C were recorded at a distance of 5, 10 and 15 mm respectively. Confocal microscopy images demonstrated the geometry of the ablation zone at different depths throughout the tumour phantom.

This study reports a novel cancer cell-loaded phantom model capable of generating real-time temperature data and visualising ablation zones during microwave ablation. This phantom model is a useful tool for clinicians to more accurately predict ablation boundaries in terms of power and timing settings, using a clinically relevant sized phantom model for the treatment of lung cancer and other cancers.

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Two-step crosslinking approach for development of photocrosslinkable biomaterial inks for extrusion-based 3D bioprinting

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INTRODUCTION: Bioprinting applications are expanding in basic tissue engineering, regenerative medicine, personalized medicine, and organ-on-chip technology. Bioink plays an important role in fabricating suitable 3D scaffolds and is crucial to achieving physiological relevance in in vitro models. The versatility and ease of use of photocrosslinkable bioinks have sparked interest in the field of 3D bioprinting. An ideal bioink should possess the desired physicochemical properties, such as proper mechanical, rheological, chemical and biological characteristics. The selection of the printing technique depends on the biopolymers, crosslinking chemistry of the bioink, and size and complexity of the intended scaffold. We have developed Gellan gum (GG) [1], Gelatin [2] and hyaluronic acid [3] -based biomaterials inks utilizing a two-step crosslinking approach. In all the cases, we achieved excellent printability by utilizing a two-step crosslinking approach: ionic and metal chelation at the first step to control the ink viscosity, followed by photo-crosslinking after the printing.

RESULTS & DISCUSSION: Natural polymers, such as polysaccharides and polypeptides, are favoured for 3D cell culture because they can support cell growth and differentiation. However, they lack the necessary rheological properties for 3D printing. In this work, we have introduced a two-step crosslinking approach using non-covalent interactions for pre-crosslinking, followed by photocrosslinking. By varying the crosslinking type, this method enables the adjustment of hydrogel properties to suit specific application requirements for extrusion-based printing. These non-covalent interactions include temperature, ionic crosslinking, metal coordination, and pH modulation (Figure 1, i–iv).

Gelatin (Gel), Gellan gum (GG) and Hyaluronic acid (HA) are chemically modified to GelMA/GelMA-GA, GGMA, and HAMA/HAGA. Gelatin-based hydrogels can be temporarily crosslinked through cooling, while GG-based hydrogels can be crosslinked via ionic interaction in the presence of divalent ions. To achieve metal chelation and pH modulation, gelatin and HA were grafted with gallic acid (GA). The UV-Vis and 1H-NMR spectroscopy confirmed the degree of methacrylation and conjugation of GA to the polymer backbone. The printable inks were achieved by pre-crosslinking, pre-characterized via rheology, and then printed into multi-layered grid structures, as shown in Figure 1 (i–iv).

Results show that ionic pre-crosslinkers offer the highest printing resolution, followed by temperature, metal chelation and pH modulation. In summary, the two-step crosslinking technique improves hydrogel printability and stability. Moreover, the developed two-step strategy can be easily adapted to create a library of bioinks.

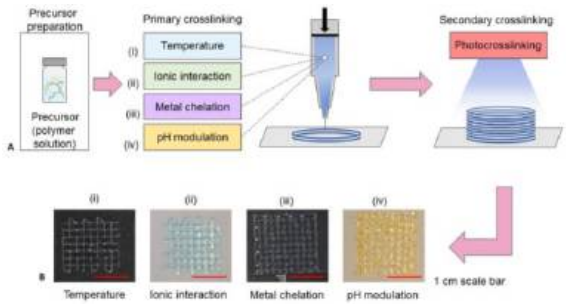

Fig. 1: (A) General strategy for two-step crosslinking utilized in this work for extrusion-based bioprinting. (B) Printing results using different pre-crosslinking techniques: (i) GelMA, (ii) GGMA, (iii) GelMA-GA, and (iv) HAMA-HAGA.

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ACKNOWLEDGEMENTS:

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Condensed Orchestration of Mesenchymal Stromal Cells via Dynamic Temperature-Actuated Hydrogels

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INTRODUCTION: Substantial bone defects' management traditionally relies on autologous bone grafts, a practice burdened with donor site morbidity and constrained supply complications [1]. Endochondral ossification, the mechanism of forming long bones, hinges on the aggregation and proliferation of precursor cells along the bones' longitudinal axis [2]. Replicating this process in vitro necessitates orchestration and maintenance of cell alignment and proximity, which has been challenging due to the cell tendency to migrate in random directions and the lack of dynamic signals which could maintain their alignment. To address this challenge, we developed a thermoresponsive hydrogel by combining polyacrylic acid microgels (Carbopol® 940 (CP)), gelatine and poly(N-isopropylacrylamide)-chondroitin sulfate (pNIPAAM-CS). The thermoresponsive nature of pNIPAAM allows a transition between hydrophilic and hydrophobic states upon cooling below or heating across the critical solution temperature of 32°C, respectively [3]. In this study, we explored 3D printing of human mesenchymal stromal cells (hMSCs) in columnar channels within this hydrogel system. By hypothesizing that the temperature-responsive environment could potentially control cell attachment and detachment, we utilized temperature actuation to induce and maintain the development of the oriented cellular channels.

METHODS: Human bone marrow-derived mesenchymal stromal cells (MSCs), as single cells or pre-aggregated into 200,000-cell pellets, were suspended in 6% w/v porcine gelatine at $4x10^6$ cells/mL. Extruded through a 300 µm needle into 1 mL of a thermoresponsive hydrogel (3% pNIPAAM-CS, 0.8% CP, 1% gelatine), constructs were cultured for 5 weeks under static (37°C) or temperature-actuated conditions (from 37°C to 25°C for 15 min every 5 days). Phalloidin and DAPI stainings enabled visualization, while ImageJ facilitated semi-quantitative analysis (minimum 11 measurements per time point from three hydrogels).

RESULTS: During a 35-day culture under static conditions, there was no significant change in the pellet's shape or length ($p > 0.48$). However, under dynamic temperature-actuated conditions, the pellets reorganized into columnar-like structures. The roundness of the pellets decreased from 0.77 to 0.46 (pellet elongation) from day 1 to day 7 ($p < 0.0001$). By day 35, there were further changes, with the cellular patterns elongating from 206 µm to 586 µm ($p < 0.0001$), which was due to both individual pellet elongation and some pellets fusing together.

DISCUSSION & CONCLUSIONS: Owing to the thermoresponsive properties of pNIPAAm, we posit that the observed cellular behavior under temperature-actuated culture conditions is associated with the periodic detachment of cells from the surface of embedded longitudinal channels. This phenomenon likely minimizes the dispersion of cells and associated proteins. Consequently, it sustains the geometric integrity and alignment of the printed patterns, facilitating the spatial cohesion of pellets and their autonomous elongation along a shared axis. This paradigm presents promising prospects for enhancing the organizational characteristics of engineered tissues.

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Xeno-free and photocurable nanocellulose-based macroporous-gel bioprinting in service of 3D cell culture

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INTRODUCTION: In the context of three-dimensional (3D) cell culture, 3D printing is a powerful tool for customizing *in vitro* 3D cell culture models that are critical for understanding the cell−matrix and cell−cell interactions. Cellulose nanofibril (CNF) hydrogels are emerging as a highly functional xeno-free and non-ECM component in formulating bioinks and constructing scaffolds able to imitate tissue in a microenvironment. Bioinks formulations that contain methacrylated biopolymers are prevailing in 3D bioprinting. To better fit nanocellulose into the bioprinting work, we customize the synthesis of methacrylate cellulose nanofibrils (CNF-MA) to yield a transparent and photocurable nanomaterial hydrogel at a low concentration less than 1 wt%[1]. To efficiently accommodate the cellular activities within a macroporous hydrogel, microgel-based strategy of CNF-MA is exploited with extrusion fragmentation and 3D bioprinting strategy is further established in the context of hydrogel-extrusion 3D printing to fabricate 3D cell culture model with microgels of CNF-MA.

METHODS: Synthesis of CNF-MA and copolymerization of CNF-MA-PAA; Hydrogel material property and microgel assembly of CNF-MA and CNF-MA-PAA; 3D bioprinting protocols for 3D culture of fibroblasts, pancreatic cancer cells, and human umbilical vein endothelial cells (HUVECs); Assessment of cellular activities within the 3D cell culture model.

RESULTS:

Through photo-induced copolymerization of CNF-MA in presence of acrylic amide monomer, hybrid composite hydrogel of natural CNF-MA with polyacrylamide (CNF-MA-PAA) is extended to modulate the matrix stiffness of the hybrid microgels. Successful utilization of CNF-MA in extrusion-bioprinting enpowered with microgel assembly provides interconnected macroporosity in 3D bioprints to promote the cellular activities of laden cells.

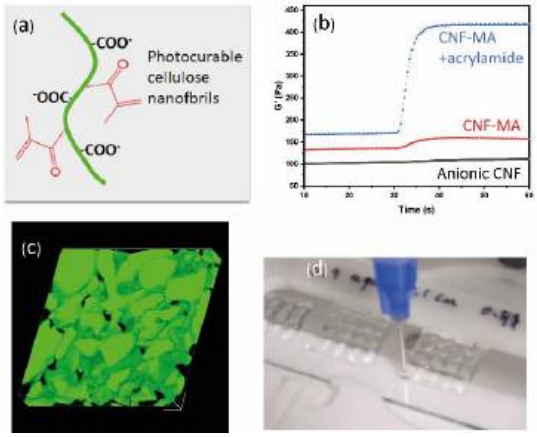

Fig. 1: (a) Surface functional groups on CNF-MA; (b)Photo-crosslinking of CNF-MA alone or with acrylamide; (c) Confocal image of the microgel of CNF-MA-PAA (scale bar:150 μm); and (d) Extrusion 3D bioprinting of CNF-MA microgels

DISCUSSION & CONCLUSIONS: Xeno-free and photocurable nanocellulose bridges the renewable natural resources, towards sustainable biomaterials, creating added-value nanomaterials derived from botanical biomass.

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ACKNOWLEDGEMENTS: Business Finland is acknowledged for financial support to the RtoB project of 3D CelluGel - Novel Bioinks 100% Based on Finnish Trees' (1529/31/2022) at Åbo Akademi University.

Tuning the assembly of ureido-pyrimidinone molecules in living systems

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INTRODUCTION: Molecular self-assembly is defined as the spontaneous organization of molecules into structurally well-defined aggregates through non-covalent interactions. It plays a key role in cellular life maintenance due to the dynamic nature of the interactions involved.

2-Ureido-4[1*H*]-Pyrimidinone (UPy) molecules are synthetic supramolecular monomers capable of forming pH responsive fibers and hydrogels in water through a combination of directional hydrogen bonding, π-π and hydrophobic interactions. Despite their wide use as biomedical materials, poor investigation of the structure-assembly relationship has been conducted so far [1]. Hence, the current project aims to elucidate the monomer structural features needed to induce the self-assembly of UPy molecules in aqueous environment by systematically modifying the monomer hydrophilic-hydrophobic balance.

Furthermore, supramolecular chemistry has gained increasing interest as a chemical tool to steer and control the cell fate intracellularly during the past years. Exemplary is the recent technology to induce assembly of certain peptide sequences in living cells upon exposure to specific biological stimuli [2]. Hence, the second goal of this project is to combine the aforementioned strategy with the self-assembly of UPy molecules to obtain spatial control over the assembly process in living systems.

METHODS: A library of UPy molecules was synthesized and fully characterized. Their assembly behavior was analyzed via a plethora of spectroscopic, scattering and imaging techniques such as Nile Red assembly assay, SAXS, UV-vis, ^{1}H-NMR, HDX-MS and CryoTEM. Next, one UPy molecule was modified with a redox responsive boronic acid cage (BA) on the terminal carbonyl group of the UPy ring in order to trigger its assembly in the cytosol of cancer cells upon exposure to high hydrogen peroxide concentration. The modified boronic acid-UPy molecule (BA-UPy) was characterized through the same techniques reported above. Additionally, the hydrogen peroxide mediate de-boronation was verified via LC-MS. Finally, the modified molecule was complexed with a cell-penetrating peptide (SHA-TAT) through a dynamic covalent bond to induce cell internalization and the cellular response was screened via toxicity and cell-internalization experiments.

RESULTS: A library of UPy molecules with different alkyl spacer and polyethylene glycol (PEG) length was synthesized. As expected, a decrease in hydrophobicity leads to more dynamic assemblies in water such as micelles and small fibers, whereas more hydrophobic molecules form more robust and stable aggregates such as double fibers. Furthermore, the presence of a urea group embedded into the hydrophobic pocket was shown to enhance the 1D-fiber formation. On the other hand, the modification of one of the more robust UPy molecule led to the formation of smaller assemblies upon complexation with the SHA-TAT peptide which could get internalized by cells. The complex presents a more toxic effect compared to the bare UPy and the BA-UPy. However, at the used concentration, the de-boronation does not occur in living cells.

DISCUSSION & CONCLUSIONS: A detailed investigation of the structure-assembly relationship of UPy molecules highlighted the importance of the alkyl spacer length and the presence of an embedded urea group to promote the 1D-fiber formation. As a result, only the UPy molecules with longer alkyl spacers and a urea group within it form stable structures such as ribbons and double fibers. In addition, the BA-UPy molecule can form assemblies in aqueous environment which can get internalized by cancer cells upon complexation with the SHA-TAT peptide and the resulting complex was shown to exert a more pronounced toxic effects compared to the bare UPy and BA-UPy molecules. However, at the highest concentration tested in cells, de-boronation was not observed.

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NAD-boosting nanoparticle drug delivery system for glaucoma treatment

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INTRODUCTION: Glaucoma is a common age-related progressive optic neuropathy characterized by the progressive death of retinal ganglion cells. It is currently the leading cause of irreversible blindness that is projected to affect ~112 million people worldwide by 2040 [1]. Current therapies have been proven insufficient and therefore there is an urgent medical need for alternative therapeutic strategies for glaucoma. Recently, the decrease of the metabolic intermediate nicotinamide adenine dinucleotide (NAD) has been associated to glaucoma pathogenesis. Thus, NAD production is an ideal target for neuroprotective therapies of glaucoma [2]. It has also been shown that natural antioxidants (green tea polyphenols) can boost NAD production. However, they suffer from poor bioavailability and stability that limit their effectiveness.

Taking these into consideration, here, we propose a novel drug delivery system based on biocompatible nanoparticles for eye delivery of natural antioxidants that boost NAD production (Fig. 1). Nanoparticles can be used as effective drug carriers for eye delivery, because of their high surface-to-volume ratio, that enables high drug loading values [3], with enhanced drug bioavailability.

METHODS: We have synthesized silica (SiO_2) nanoparticles by flame spray pyrolysis (FSP) that is a highly reproducible and inherently scalable technique. The as-synthesized SiO_2 nanoparticles were first functionalized by 3-aminopropyl-triethoxysilane (APTES) and then natural antioxidants, i.e. gallic acid (GA) and epigallocatechin (EGCG), were covalently grafted on their surface. Kinetic experiments based on the 2,2,-diphenyl-1-picrylhydrazyl (DPPH) radical method were used to assess the radical scavenging capacity of the nanoformulations. The NAD-boosting capacity of the nanoformulations was evaluated *in vitro* by luminometry NAD assay on cortical neurons in suspension.

RESULTS: The produced nanoparticles had a specific surface area of 216 m^2/g (primary particle size of 10 nm). Hight GA and EGCG loading values were achieved (90 mg GA/g SiO_2-APTES and 150 mg EGCG/g SiO_2-APTES). DPPH assay verified that GA and EGCG maintained their radical scavenging capacity upon loading on the nanoparticles.

Luminometry NAD assay showed a higher NAD fold change for GA and EGCG loaded SiO_2 nanoparticles compared to SiO_2 nanoparticles at equivalent particle concentration.

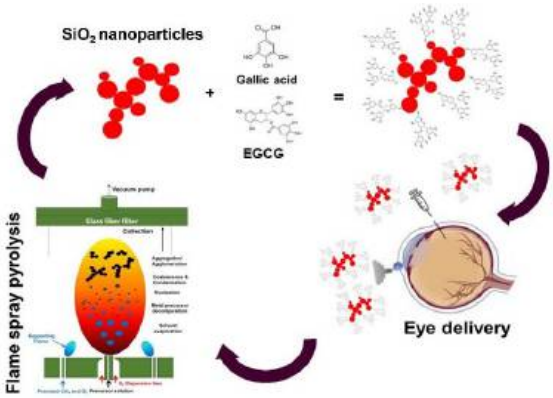

Fig. 1: Schematic concept of the present study.

DISCUSSION & CONCLUSIONS: Our results imply that flame-made nanocarriers with high drug loading capacity have the potential to improve eye delivery of antioxidants for glaucoma prevention. As the proposed drug delivery system consists of biocompatible and natural compounds, the translation of the proposed formulation, even in societies that lack adequate ophthalmic care can be promoted.

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Novel injectable chitosan methacrylate (ChiMa) hydrogel reinforced with oxidized cellulose nanofibers methacrylate (OCNFMA) for hemostatic and wound healing agents

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INTRODUCTION: Composite materials with excellent biocompatibility, degradability, and controlled drug release are essential for tissue engineering[1-4]. Several natural polymers, such as cellulose nanofiber (CNF)[1] and chitosan (Chi)[2], have been widely used in medical applications, such as hemostatics and wound healing. CNF derived from plants and chitosan (Chi), which are derived from deacetylated crustaceans and insects, are both being used in biotechnological, medical, and cosmetic applications[4]. Polymers such as CNF and Chi can be methacrylated to prepare injectable photocurable hydrogels for treating wounds[2]. In this study, ChiMA-CNFMA hydrogels were combined in different ratios to create an injectable *in situ* hydrogel system for hemostatic and wound healing applications.

METHODS: In this work, the 2 wt.% of CNF was subjected to oxidation using varying amounts of sodium periodate in order to achieve different levels of oxidation. The solution was dialyzed for 4-5 days with a 3500kDa dialysis tube to remove IO_3. The modified CNF and Chi were subjected to a methacrylation process using 2-aminoethyl methacrylate hydrochloride (AEMA) and methacarylic anhydride (MA)[2] to prepare CNFMA and ChiMA. ChiMA-OCNFMA hydrogels were prepared at different weight ratios (3:7, 5:5, and 7:3) and their properties were compared with those of pristine ChiMA. The hydrogels were crosslinked with UV in the presence of 0.3 wt.% lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP). We examined the hydrogel systems with scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and rheological characteristic. To determine their potential for biomedical application, their antibacterial and blood clotting indexes, as well as their cytotoxicity, were evaluated.

RESULTS: The successful syntheses of OCNFMA and ChiMA were confirmed by FTIR. The OCNFMA-CHIMA composite hydrogels reached complete gelation in 1 min.

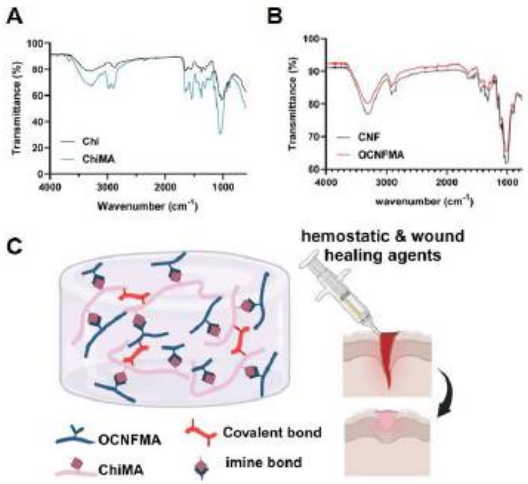

Fig. 1: FTIR analysis of A) Chi and ChiMA; B) FTIR analysis of CNF and OCNFMA; and C) schematic illustration of a dual crosslinking hydrogel based on OCNFMA and ChiMA for hemostatic and wound healing purposes.

DISCUSSION & CONCLUSIONS: Hydrogels with antibacterial and hemostatic properties have shown promising potential as hemostasis and wound healing agents. This composition could also be used as a promising bioink for the 3D printing of tissues and organs.

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Multifunctional nanocellulose-based hybrid hydrogel wound dressing for wound infection treatment

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INTRODUCTION:
Wounds disrupt the natural skin barrier, facilitating pathogen penetration and increasing the risk of infections. Wound infections are a major healthcare concern and can result in patient morbidity and mortality, as well as a substantial cost for the healthcare system. The problem is exacerbated by the alarming increase in multi-drug resistant wound pathogens. We present a hybrid hydrogel wound dressing that can release high concentrations of antimicrobial peptides (AMPs) that are efficient against clinical isolates of multidrug resistant bacteria.

METHODS: The designed AMP *D*-6-C5-Leu [1] was loaded in mesoporous silica nanoparticles (MSNs) using a combination of impregnation and slow-drying. The AMP loaded MSNs were mixed with BCN-modified hyaluronic acid (HA) and azide-modified protease degradable cross-linkers to form hybrid hydrogels. The hydrogels were integrated unilaterally with bacterial nanocellulose (BC) wound dressings by mechanical interlocking (Fig. 1).

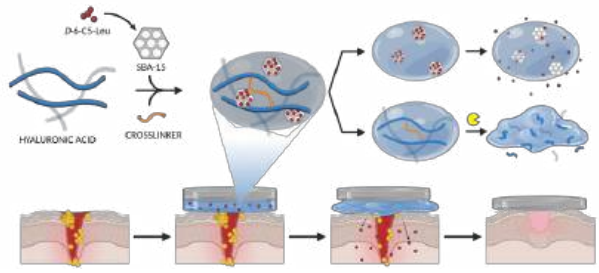

Fig. 1: Preparation of BC-HA-AMP@MSN hybrid wound dressing.

RESULTS: A bilayer hybrid wound dressing material was obtained by grafting HA hydrogels on BC with controllable thickness, varying from a few hundred µm to 1-2 mm. The hydrogels were crosslinked using a peptide crosslinker susceptible to degradation by several matrix metalloproteinases and bacterial proteases. Hydrogel degradation was demonstrated using collagenase type 1 (Coll-1). A Coll-1 concentration of 0.005 mg/mL resulted in ~20 % hydrogel degradation in 8 hours whereas 0.5 mg/mL Coll-1 resulted in complete dissolution of the hydrogels. The degradation of the hydrogels resulted in release of low molecular weight HA that can promote wound healing. In addition, the degradation promoted the release of AMP loaded MSNs. Due to the small mesh size of the hydrogels (< 60 nm), the MSNs (SBA-15) were efficiently retained in intact hydrogels. The number of MSNs could be tuned by varying either their concentration and/or the thickness of the HA hydrogels. A higher number of MSNs resulted in a higher dose of AMPs. An AMP loading of about 200 mg/g of MSNs was achieved, however, due to the high loading capacity of SBA-15 the AMP loading could be further enhanced. Encapsulation of AMP@MSN in the HA matrix improved the mechanical properties of the hydrogel without any negative impact on the flexibility and conformability. In addition to promote a high dose and steady AMP release, the MSNs effectively prevented proteolytic cleavage of the AMPs. The antimicrobial effect of the dressings was confirmed *in vitro* against *S. aureus*.

DISCUSSION & CONCLUSIONS: The wound dressing showed good conformability, full transparency, and adequate moisture retention. The protease degradable HA hydrogel grafted to BC can efficiently trap large quantities of AMP loaded MSNs, providing a reservoir for localized delivery of antimicrobials. A high AMP loading and release efficiency results in high local dose and a high bactericidal effect, which can facilitate treatment of infected hard to heal wounds.

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Loading and coating of bacterial compounds into/onto electrospun nanofibers for biomedical applications

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INTRODUCTION: Probiotics are known as live microorganisms with the potential to confer a health benefit to the host [1]. Not only probiotics are available as commercial products and dietary adjuncts, but also probiotics have been extensively studied as a treatment supplement for various diseases, such as diarrhea, irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) [2,3]. Probiotics need to be metabolically stable and survive the passage through the stomach to reach the intestines, and then confer health- enhancing proprieties. Oxygen, pH, and storage temperature are the main factors affecting the viability of these microorganisms [1]. It has been shown that encapsulation of the bacteria in a polymer matrix may lead to enhanced delivery of probiotics thanks to the physical barrier provided [4]. Among different encapsulation methods, electrospinning has shown great potential for enhanced delivery of probiotics. However, it has been mainly a mono-layer electrospun layer [5]. We have recently focused on further improvement of probiotics viability for enhanced delivery via encapsulation into multilayer nanofibers, or coating bacteria onto electrosun sheets.

METHODS: We designed and fabricated a three-layer construct consisting of an inner hydrophilic and water-soluble layer (pullulan) for safe encapsulation of probiotic cells (spray-dried Lactobacillus rhamnosus GG (LGG)), and two outer hydrophobic layers (PLGA) to improve the storage potential. We compared the viability of the fabricated construct with the probiotic (powder form) via *in vitro* and *in vivo* competition study, and further investigated the fiber-bacteria interaction via external exposure.

RESULTS: We demonstrated that the three-layer construct, where LGG:pullulan electrospun layer is sandwitched between two electrospun PLGA layers represents higher survival and enhanced storage of LGG compared with spray-dried LGG and the monolayer of LGG:Pullulan. Also, the LGG, delivered by nanofibers, were able to survive intestinal transit and were recovered from all segments of the gut. According to our *in vivo* assay using LGG, nanofibers provide similar seeding as spray-dried powder delivery and even better colonisation at some GIT locations. We further demonstrated that adhesion pattern of probiotics to nanofibers of different polymers is not similar and requires further assessment.

DISCUSSION & CONCLUSIONS: The layered electrospun fiber technology represents an interesting alternative for the protection and delivery of viable probiotics to the intestine. The hydrophobic external layer assisted with improved stability and protection against the immediate release of probiotics into the stomach, and the extracellular matrix (ECM)-like structure of nanofibers increased the muco-adhesive properties of the construct. Both forms of bacteria as encapsulated or coated are worth further attention for use in next generation probiotics.

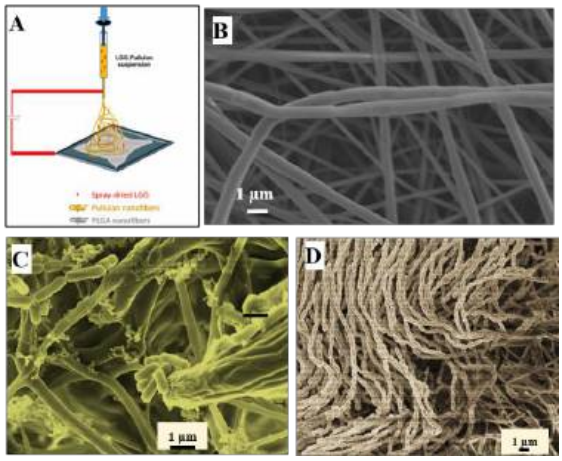

Fig. 1: schematic representation of a sequential electrospinning to encapsulate bacteria (A); and encapsulation (B) and coating (C,D) of LGG bacteria into/onto electrospun nanofibers .

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Enzymatically Triggered Deprotection and Cross-Linking of Thiolated Polymers for 3D Cell Culture and Bioprinting Applications

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INTRODUCTION: The development of tissue and disease models using human cells in an extracellular matrix (ECM) mimetic environment holds great promise for drug development, tissue engineering, regenerative medicine applications, and can provide a better understanding of cell-matrix interactions. Currently, cells and organoids are primarily cultured on matrices extracted from animals, such as the basement membrane from the Engelbreth-Holm-Swarm mouse tumor (EHS-gel). However, aside from ethical issues and variations between different batches, the complex nature of these materials hinders researchers from systematically studying cellular responses to changes in their microenvironment. Modular hydrogels offer a solution by providing a well-defined environment where different characteristics, such as stiffness and biofunctionality, can be independently tuned. Development of modular hydrogels relies on bioorthogonal chemistries that provide controlled hydrogel formation and modification in the presence of cells.[1]

METHODS: We have developed an enzyme-responsive chemistry that allows us to regulate the initiation of hydrogel formation. Thiols protected with the S-Phenylacetamidomethyl (Phacm) protection group were conjugated to various polymeric backbones, including polyethylene glycol (PEG-SP) and polysaccharides such as alginate (AlgCP). We explored the mechanical properties, fine-tuning stiffness, printability, and biocompatibility of these hydrogels. Different cell types, such as MCF7 breast cancer cells, primary human dermal fibroblasts, and murine intestinal crypts, were cultured using these hydrogels.

RESULTS: As illustrated in Figure 1, upon the addition of penicillin G acylase (PGA), thiols undergo deprotection and initiate reactions, either forming disulfides by reacting with other free thiols [2] or to thiol reactive molecules such as maleimides, [3] to crosslink the hydrogel. We demonstrated the dynamic softening of the hydrogels using glutathione (GSH) as a reducing agent in the presence of cells.[3]

Furthermore, we successfully bioprinted MCF7 breast cancer cells and primary human fibroblasts using these hydrogels, demonstrating high cell viability[2,3] The potential for bioprinting multi-material and multi-cellular structures was also explored.[2,3]

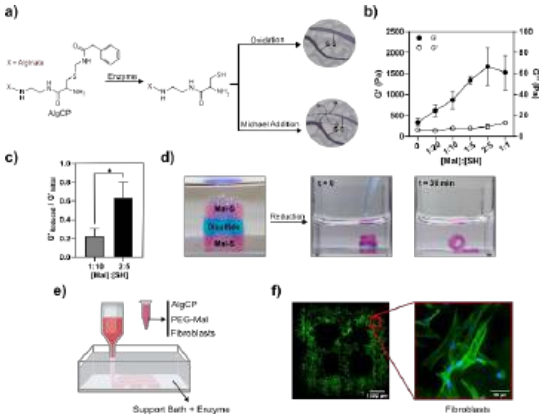

Fig. 1: a) Schematic demostration of enzymatically triggered deprotection and cross-linking of AlgCP.b) Tunning the mechanical properties. c) Tunning the softening of AlgCP after GSH treatment. d) A multi-material printed structure split in half in a reduced environment. e)Bioprinting setup. f)Bioprinted fibroblast cells.[3]

DISCUSSION & CONCLUSIONS: The results of this research highlight the potential of employing enzymatic deprotection of thiols to create modular hydrogels suitable for 3D cell culture and bioprinting applications. Our findings suggest that this is a biocompatible and bioorthogonal chemistry, ideal for fabricating complex structures with diverse cell types and materials. This novel chemistry presents a promising alternative to current animal-based hydrogel systems for 3D cell culture, offering increased control over cell properties.

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ACKNOWLEDGEMENTS: We gratefully acknowledge financial support from the Swedish Government Strategic Research Area in Materials Science on Functional Materials at Linköping University, the Carl Tryggers Foundation, and the Knut and Alice Wallenberg Foundation.

Biopolymer-based inks for 3D printing

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INTRODUCTION: Biopolymers are abundant, generally biodegradable and non-toxic, and therefore particularly well-suited for biomedical applications. Hydrogel formation, antimicrobial- and immunogenic activity, water-retention, and shear thinning are examples of characteristics making biopolymers attractive as biomaterials. These are also properties that make biopolymers suitable as inks for 3D printing [1]. 3D printing enables the preparation of pre-determined porous structures using for example a micro-extrusion system. Chitosan is a cationic polyelectrolyte prepared by de-*N*-acetylation of chitin, a structural biopolymer found in the exoskeleton of insects and crustaceans. Chitosan is known to have antimicrobial properties and has been suggested as an interesting material for 3D printing of scaffolds acting both as a structural and bioactive component [2]. Nanocellulose is another biopolymer extensively researched for the preparation of shear thinning inks with the capability of printing high resolution constructs [1]. Inks are often prepared by combing several polysaccharides, for example with the aim of printing a 3D bioconstruct with controlled architecture, strength, porosity, and bioactivity. In this work, TEMPO-oxidized nanocellulose (TO-CNF) and chitosan were studied for 3D printing applications. Additionally, alginate was added to the TO-CNF to tune the Ca^{2+}-binding of the inks.

METHODS: TEMPO-oxidized cellulose nanofibers (2 %) with two different charge contents were prepared. The carboxylic acid content was 1.4 mmol/g (low charge, LC-CNF) and 1.8 mmol/g (high charge, HC-CNF). Inks were prepared with and without the addition of alginate (20 % by weight) (LF 10/60). A Regemat3D printer was used. Cross-linking was caried out after printing with $CaCl_2$ (50 mM). The constructs were then freeze-dried, and immersed in water. Images were obtained after printing, after cross-linking and after freeze drying using a scanner to evaluate the shrinkage. Chitosan inks were prepared from samples having different M_w and relatively constant DDA, dissolved in acetic acid. The constructs were frozen between the extrusion of each layer, and immersed in NaOH before drying (either simply in air or freeze drying).

RESULTS: LC-CNF and HC-CNF could be printed using the extrusion printing principle (Fig. 1). For the HC-CNF high resolution constructs could be obtained. The cross-linking was achieved by the additions of Ca^{2+}, and 8 % shrinkage was quantified after cross-linking. Additional shrinkage (4 %) resulted from drying, before the constructs swelled 2 % upon immersion in water. No shrinkage upon the addition of Ca^{2+}, and reduced shrinkage upon drying (< 4 %) was obtained for the LC-CNF. Upon the addition of alginate, the shrinkage of both samples increased significantly when Ca^{2+} was added; 8 % and 14 % for LC- and HC-CNF, respectively. For chitosan, increased resolution was achieved by freezing each layer of a grid construct during extrusion. Shrinkage (10 – 15 %) occurred upon drying.

Fig. 1: 3D printing of a flower using TEMPO-CNF ink.

DISCUSSION & CONCLUSIONS: This work demonstrates that inks prepared from TEMPO-CNF cross-linked with Ca^{2+} are well suited for 3D printing applications. The Ca^{2+}-binding capacity of the ink, and hence, the strength of the resulting construct could be tuned by varying the degree of oxidation. Furthermore, alginate could be added to the ink to increase the Ca^{2+}-binding. The higher binding resulted in increased shrinkage during cross-linking. Thus, both strength, structure, surface properties and swelling should be considered when tailoring the composition of the inks. In the case of chitosan, high resolution could be achieved when each extruded layer was frozen before printing of the subsequent layer. Taken together, high resolution constructs could be prepared from biopolymer-based inks, adding to the potential of 3D printing for future biomedical applications.

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ACKNOWLEDGEMENTS: Chitinor AS is kindly thanked providing the chitosan samples. Thanks to the Research Council of Norway for funding (bioMAT4EYE project, Grant no. 337610)

Multi-material printing of bio-hybrid systems

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INTRODUCTION: Multi-material micro-extrusion 3D printing has opened new opportunities for merging living cells with engineered functional materials, to unlock unique applications such as of human tissue bio-hybrid implants and instrumented laboratory models [1]**.** Across these applications, a core challenge lies in the development of biocompatible functional materials inks that can be seamlessly integrated with living tissue. Towards this goal, we are developing soft, biocompatible electronics, integrative bio-printing methodologies, and tissue-instructive matrices.

METHODS: Elastomeric Styrene-Ethylene-Butylene-Styrene (SEBS) inks and conductive composites based on these, were created using butyl acetate as carrier solvent as described previously [2]. Bio-electronic tissue-culturing devices were created by integrating several SEBS inks on a multi-material micro-extrusion 3D printing platform (3D discovery, RegenHU, CH). Bio-printed tissues were created using embedded printing into alginate slurries, and cell-instructive and transparent nano-fibrillar cellulose inks as described previously [3,4].

RESULTS: By combining several SEBS composite inks, we can create soft electronic culturing devices comprising single-micron and free-standing features as well as devices for monitoring and stimulating electrically and mechanically active tissue models, see Fig1.

We merge these devices with bio-printed muscular and neuronal tissue by means of by embedded printing into granular gel composites. Using supports that contain ECM components, we create dynamic environments that facilitate 3D outgrowth of neuronal axons as well as cell migration [2]. For fully defined, cell-guiding supports we apply customized nano-fibrillar cellulose with tailored cell binding peptides. Shear-induced alignment of fibrillary content during printing serves as a facile methods of creating anisotropic striated muscle tissue models. We are currently exploring the use of integrated electrical modalities for stimulating and monitoring tissue development.

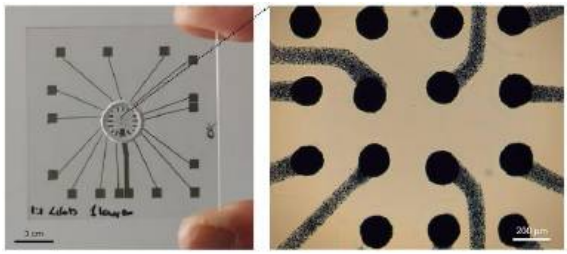

Fig. 1: Fully printed multi-electrode array dishes

DISCUSSION & CONCLUSIONS: Traditional bio-electronic devices derived from clean-room lithography are inherently ill-suited for integration within soft, dynamic and three-dimensional engineered tissues. Multi-material 3D printing of soft electronics materials provides and appealing alternative, at the expense of less spatial resolution and lower electronic fidelity. Still, as supported by our work, multi-material micro-extrusion 3D printing has unique capabilities for integrating synthetic and living matter on a single platform. By continuously improving 3D printing methodologies and materials we propose that true bio-hybrid manufacture of biologically accurate tissues with integrated functional modalities will be realized within a foreseeable future.

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ACKNOWLEDGEMENTS: The research was performed by current and prior members of Tailored Materials and Tissues at DTU: https://www.healthtech.dtu.dk/tailored-materials-and-tissues.

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Self-Assembly Driven Biomimetic 3D-Printed Hybrid Aerogel-based Theragenerative Scaffolds for Bone Regeneration and Bone Cancer Therapy

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INTRODUCTION: Due to the clinical limitations in both tumor therapy and bone regeneration[1], the development of novel approaches based on bioactive theragenerative materials (combining therapy and regeneration) is of outmost importance and need. In this regard, the rational design of the theragenerative[2] 3D printed smart hybrid aerogel scaffolds has shown great promise in regulating cell-matrix interactions with enhanced osteoconductivity, osteoinductivity, anti-bacterial and anti-tumoral properties.

METHOD: Profiting from the ease of surface modification of silk fibroin (SF) biopolymer and its self-assembly capability, we recently fabricated a range of 3D printed bio-inspired theragenerative aerogel-based nanocomposites with controlled microstructure, mechanical properties[3, 4, 5, 6]. The fabrication of these biomimetic scaffolds involved integrating diverse functional inorganic nanoparticles, including hollow mesoporous silica nanoparticles[3], black bioactive glass mesoporous nanofibers, titanium carbide (Ti_3C_2, MXene) nanosheets[6], and bismuth sulfite (Bi_2S_3) nanobelts[5], within ligand-modified SF. This integration facilitated the development of stable 3D printable hydrogels and aerogels. This was achieved through the self-assembly of SF, leveraging robust covalent and bio-inspired non-covalent supramolecular interactions.

RESULTS: Both the therapeutic and bone regeneration potential of developed composite aerogels have been governed by biochemical properties of loaded functional micro and nanoparticles in the aerogel network. Intriguing recent examples in this regard are the incorporation of photothermally active MXene 2D nanosheets[6] and black bioactive glass nanofibers (BGF, SiO_{2-x} 75%, CaO 25%, P_2O_5 5%) inside the SF biopolymer matrix through mussel-inspired chemistry.

DISCUSSION & CONCLUSIONS: In this study, we demonstrated that while 3D printed composite scaffolds could mediate the *in vitro* growth, proliferation, and differentiation of osteoblastic cell lines, they also showed a strong anti-osteosarcoma activity through photothermal ablation of bone cancer cells as well as photothermal antibacterial effect upon remote irradiation with near-infrared laser (NIR) photon.

The biomimetic dual functional aerogel-based hybrid 3D printed scaffolds and chosen therapeutic techniques are thought to render a significant breakthrough in biomaterials' future clinical applications.

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Hydrogel-elastomer interface engineering for cyclic mechanical stimulation of bioprinted 3D tissue constructs

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INTRODUCTION: Several platforms for the mechanical stimulation of 3D tissue constructs are available for mechanobiological studies. Flexcell's Tissue Train is a widely used platform that enables mechanical stimulation of 3D tissue constructs attached to flexible-bottom plates (BioFlex) containing an elastomeric membrane of poly-dimethylsiloxane (PDMS) [1]. A key challenge is to ensure stable adhesion of the 3D constructs to the PDMS membrane to meet the requirements for mechanical stimulation. Previous methods to improve adhesion are complex and impractical, especially for bioprinted hydrogel constructs. The aim of this study was to modify the BioFlex plates to allow cyclic mechanical stimulation of bioprinted 3D tissue constructs.

METHODS: The 6-well culture plates (BioFlex, BF-3001U) were subjected to a water vapor/oxygen plasma treatment to add hydroxyl groups to the PDMS surface. Functionalization with -NH_2 groups (amino-PDMS) was performed by incubation with (3-aminopropyl) triethoxysilane (APTES) followed by conjugation with carboxylic acid groups. For methacrylate-functionalized surfaces (meth-PDMS), immersion with 3-(trimethoxysilyl)-propyl methacrylate (TMSPMA) was performed. The modified plates were characterized by ATR-FTIR. A mixture of hydrogels (GelMA and alginate) and bladder smooth muscle cells (SMCs)[2] was printed on the modified plates using a bioprinter (Bio X, Cellink), and adhesion was evaluated over time. The effects on the expression of contractile markers were analyzed by immunofluorescence staining and semi-quantitative RT-PCR.

RESULTS: Surfaces modified with methacrylate enabled stable adhesion of the 3D constructs to the membrane and facilitated cyclic mechanical stimulation, which significantly increased the expression of contractile markers at the mRNA and protein levels. Notably, these effects were mediated through the activation of the p38 MAPK pathway, as demonstrated by the dose-dependent abolition of stimulation effects upon pathway inhibition.

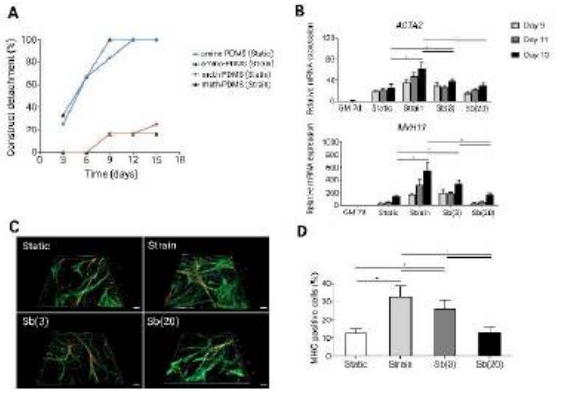

Fig. 1: ***A)*** *Comparison of adhesion tests performed on PDMS surfaces modified by different methods;* ***B)*** *qRT-PCR results for ACTA2 and MYH11 in different groups;* ***C)*** *Immunofluorescence staining of SMCs inside the bioprinted constructs in different experimental groups, (green: α-SMA, red: MHC)* ***D)*** *Quantification of MHC positive cells.*

DISCUSSION & CONCLUSIONS: This study explored two approaches for conjugating hydrogels to PDMS surfaces of BioFlex plates after water vapor/oxygen plasma treatment. Methacrylate-functionalized PDMS (meth-PDMS) demonstrated effective adhesion of hydrogels under static and dynamic conditions, allowing in-situ bonding during UV curing. In contrast, amino modification, while initially promising, failed to retain constructs under dynamic conditions, possibly due to insufficient crosslinking time chosen to preserve cell viability. The meth-PDMS modified plates allowed cyclic mechanical stimulation of SMCs, which underwent morphological changes linked to a contractile phenotype, with increased gene transcription and expression of contractile marker proteins mediated through the p38 MAPK pathway.

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ACKNOWLEDGEMENTS: This research was funded by grants from the China Scholarship Council, S.C. Van Fonden, Grosserer L.F. Foghts Fond, Simon Fougner Hartmanns Familiefond, and Brødrene Hartmanns Fond.

Fibrin Stiffness Regulates Phenotypic Plasticity of Metastatic Breast Cancer Cells

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INTRODUCTION: Triple negative breast cancer (TNBC) is the most aggressive breast cancer type with a high metastatic tendency and lack of targeted treatment [1]. To develop better treatment options, there is an urgent need to understand the mechanisms behind cancer dissemination and the role of circulating tumor cells (CTCs) in it. [2] However, most used *in vitro* models for metastasis are limited to studying invasion and migration in basement membrane extract (e.g. Matrigel) or collagen based matrices [3]. To study CTC behaviour, a matrix that models metastatic steps occurring at the interface between circulation and tissue is needed. The aim of this work is to explore phenotypic plasticity of breast cancer cells *in vitro* using mechanically tunable 3D fibrin gels.

METHODS: Soft (10 mg/mL) and stiff (30 mg/mL) fibrin gels were prepared by the enzymatic reaction between fibrinogen and thrombin. Viscoelastic properties of the gels were characterized using rheological methods. DU4475 cells (model for CTC-like TNBC cells) were cultured in fibrin gels in the presence of fibrinolysis inhibitor. For comparison, cells were cultured in liquid suspension culture and Matrigel. After 7 days, changes in cell phenotype were evaluated using microscopy techniques, RNA sequencing analysis and Western blotting.

RESULTS: Both soft (*G´* 57 Pa) and stiff (*G´* 175 Pa) fibrin gels supported DU4475 cell growth and assembly into spheroids (*Fig. 1*). The cell morphology and gene expression patterns in fibrin gels clearly differed from Matrigel and suspension cultures. Moreover, cell culture in the stiff fibrin gel induced cells to develop protrusive bleb-like structures that were motile and stained positive for actin. The formation of these protrusions was reversible upon fibrin softening.

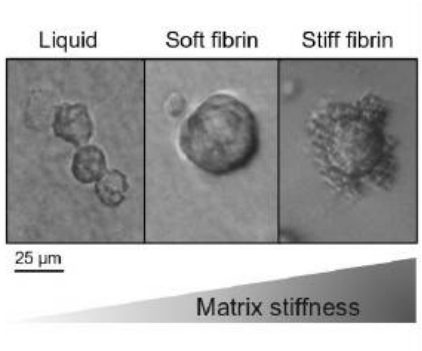

Fig. 1: Spheroid and protrusion formation in response to matrix stiffness [4].

DISCUSSION & CONCLUSIONS: Fibrin gels show excellent mechanical properties and promote cell growth in 3D breast cancer cell culture. The relationship between fibrin stiffness and protrusion formation demonstrates the importance of mechanoregulation for the phenotype of CTC-like TNBC cells. Altogether, fibrin gels represent a promising platform to study cancer cells and their adaptive responses to matrix cues.

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Bioactive Tissue Scaffolds from Decellularized Ascidian Tunic

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INTRODUCTION:

Urochordates are the closest invertebrate relative to humans and commonly referred to as tunicates, a name ascribed to their leathery outer tunic. Several ecological, economical, and public health hazards are associated with tunicates. Tunicates are 'invaders' that travel from one region or port to other by attaching to the ships; due to their high reproduction rate and temperature and salinity tolerance they can quickly replace the native species by overgrowing and taking over an area, making them a major threat to biodiversity. Harvesting the invasive tunicates for extraction of useful biomaterials offers a potential solution.

METHODS: Tunicates collected from the Zayed Port, Abu Dhabi, UAE were identified as Polyclinum Constellatum and submitted to NCBI (Accession # MW990087). The outer rough layer was removed using surgical knife; tunic tissue pieces are stirred well in decellularization buffer for 48 hours. The buffer was changed every 2 hours. The decellularized tissue pieces were frozen in -80oC overnight and lyophilized for 48 hrs. The lyophilized scaffolds were sterilized with ethanol and UV radiation for further characterization and analysis. In vitro cell culture studies using Mouse Embryonic Fibroblasts (MEFs) was performed and the cell viability was assessed using various assays.

RESULTS: We found the tunicate species with various colour morphs including brown, red, green, and honey-coloured – all belonging to the same species. This species has a jelly-like tunic consistency externally and more so internally. Predominantly made of cellulose, the cellulose fibrils are visible on slicing the top layers of the tunic. The presence of cellulose was confirmed by the material characterization experiments. The SEM images revealed that the lyophilized tunic has a rough multi-layered networked structure, with the presence of micro/nano-fibrils and crystals. It is interesting to note that the nano-fibrillar cellulose networks remain intact even after the process of decellularization and lyophilization. Tunic, being a natural ECM material, has a modulus of 4 MPa, which is much higher than other hydrogels reported so far in the literature. The tunic, derived in this work, combines both excellent biocompatibility and mechanical strength, overcoming the limitations of most of the hydrogel-based constructs.

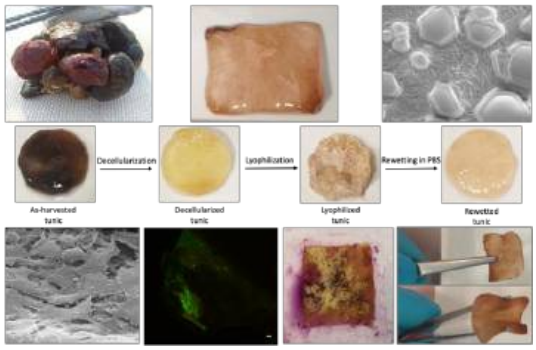

Fig. 1: Bioactive tissue scaffolds from decellularized ascidian tunic

DISCUSSION & CONCLUSIONS: In this work, we have successfully identified the environmentally destructive colonizing tunicate species of Polyclinum constellatum in the coast of Abu Dhabi and propose a method of using it as scaffolds for tissue engineering and regenerative medicine applications. Detailed morphological analysis revealed the intricate 3D nanofibrous cellulosic networks that remain intact even after the multi-step process of decellularization and lyophilization. The fact that the lyophilized tunics are dry, can be easily transported compared to other 3D culture systems such as hydrogels and on rewetting, the 3D tunic structure is regained, is a huge advantage for labs around the world trying to establish sustainable 3D culture systems. The tunic showed excellent biocompatibility, high mechanical properties (a modulus of 3.85±0.93 MPa compared to ~0.1 – 1 MPa of hydrogels) and exhibited high fluid-absorption capability. Experiments with camel blood plasma as wound exudate and alginate-based artificial wound proved the superiority of the tunic over the other commercially available wound-dressing materials, with a capacity of absorbing 20 times its weight in the dry state.

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Cost-effective antibacterial wound dressing comprising nanofibers infused with flame-made Ag/SiO_2 nanoparticles

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INTRODUCTION: The body engages in a sophisticated biological process to heal wounds, aiming to repair tissue and reinstate its structure and function. Wound infections occur when bacteria or other microorganisms enter and thrive within the wound. The presence of an infection significantly impedes the healing process leading to health risks like delayed healing, septicemia, or severe infections in deeper tissues and bones. Antibacterial dressings are specialized wound coverings infused with agents or substances having antibacterial properties. These dressings are purposefully crafted to diminish bacterial colonization in infected wounds, thus advancing the healing process. Even though antibacterial dressings are often infused with antibiotics for topical delivery, there is an ever-increasing need to develop antibiotic-free dressings, with the aim to minimise the emergence of antimicrobial resistance.

One strategy is to use antimicrobial nanomaterials, such as nanosilver, as active components in dressings. However, even though Ag-based dressings exhibit superb antimicrobial activity in pre-clinical research, commercial Ag-dressings do not exhibit clear clinical efficacy. The reason for this discrepancy is the undefined Ag^+ ion release in the wound at sub-lethal concentrations that may even promote antimicrobial resistance. Unless the production of nanosilver in those dressings is scalable and reproducible with precise nanoparticle sizes and morphology, their presence in wound dressings will mostly serve as a marketing tool rather than a functional additive. Our proposed solution involves merging two industrial manufacturing processes—flame spray pyrolysis and electrospinning—to create an antibacterial wound dressing utilizing silver-silica nanoparticles (Ag/SiO_2 NPs).

METHODS: Ag/SiO_2 nanoparticles are generated by flame spray pyrolysis, utilizing silver acetate and a combination of acetonitrile and 2-ethylhexanoic acid as precursor materials[2]. Subsequently, these particles are integrated into electrospun fibers based on a polyvinyl alcohol/chitosan (PVA/CS) composite. In this process, a blend of PVA and CS with varying PVA:CS ratios, specifically 100:0, 95:5, 90:10, and 80:20, is employed for the electrospinning procedure.

RESULTS: The nanoparticles measure between 2 and 10 nanometers in size, while the electrospun dressings exhibit a fiber diameter ranging from 100 to 400 nanometers. These dressings offer continuous release of silver ions, which leads to their antibacterial activity against Methicillin-Resistant Staphylococcus Aureus (MRSA), a pathogen recognized for its role in causing wound infections (fig 1).

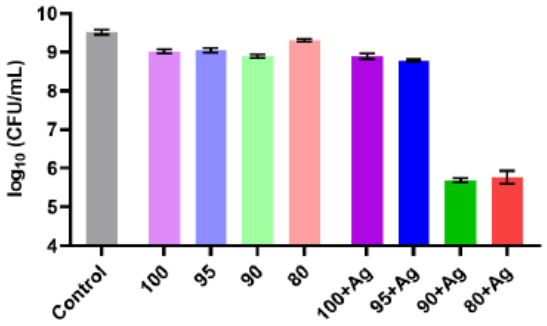

Fig 1: Antibacterial activity of electrospun membranes containing Ag/SiO_2 nanoparticles

DISCUSSION & CONCLUSIONS: This study presents an inexpensive approach for crafting wound dressings using PVA/CS nanofibrous membranes enriched with Ag/SiO_2 nanoparticles. These dressings demonstrate strong antibacterial efficacy against MRSA, particularly when using PVA:CS ratios of 90:10 and 80:20. Furthermore, the limited release of Ag^+ ions help mitigate the risk of bacteria developing resistance to silver ions.

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3D breast cancer models to study proteolytic activity *in vitro*

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INTRODUCTION: Dysregulated proteases contribute to all hallmarks of cancer [1,2]. Since protease activity is challenging to study in vivo, engineered human 3D cancer models can provide valuable insights into the complex regulation of proteases in the tumor microenvironment (TME) [3,4]. We have designed peptide-based fluorescence resonance energy transfer (FRET) sensors and modular and printable hydrogels that facilitate systematic investigation of various factors in the TME that may influence protease activity and explored the effects of proteases on breast cancer (BC) development and progression in vitro.

METHODS: Alginate (Alg) and hyaluronan (HA) were modified with bicyclo[6.1.0]nonyne (BCN) and cross-linked by strain-promoted azide-alkyne cycloaddition (SPAAC) using azide-functionalized cross-linkers. Both protease-degradable peptide-based cross-linkers and non-degradable PEG-based cross-linkers were used. BC cells (MCF-7 and MDA-MB-231) were cultured in hydrogels with different stiffness, cross-linking topology, and susceptibility to proteolytic degradation for up to 14 days. 3D bioprinting of TME models was carried out using a Cellink BioX. A peptide-based FRET sensor was designed to investigate the activity of matrix metalloproteinase (MMP) 1, 2, 3, 7, and 9.

RESULTS: BC cells cultured in non-degradable hydrogels assembled into spheroids, irrespective of stiffness. The morphology of BC cells in soft degradable hydrogels showed an elongated morphology. Inhibition of MMPs resulted in spheroid formation irrespective of the cross-linker used, indicating that proteolytic activity facilitates cell spreading and adaptation of a migratory phenotype. BC cells cultured in the stiffer degradable hydrogels also formed spheroids in the absence of protease inhibitors. 3D bioprinting of a BC niche with a separate fibroblast compartment showed the contribution of fibroblasts to hydrogel degradation and BC proliferation while no effects on BC proliferation were seen in non-degradable hydrogels. FRET sensors can further facilitate the investigation of protease activity.

DISCUSSION & CONCLUSIONS: Biofabricated BC models based on tuneable hydrogels and FRET-based sensors can offer new insights into the role of dysregulated protease activity in BC development and progression. This approach may facilitate the development of personalized cancer diagnostics and therapies targeting proteases.

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Investigating the effect of conjugation chemistry on the bioactivity of immobilized host defense peptides

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INTRODUCTION: Host defense peptides (HDPs), naturally occurring polypeptides with sequences of 12-50 amino acids, are becoming highly interesting as a new generation of therapeutics for a wide range of applications such as wound healing, cancer treatment and antimicrobial therapy [1]. However, their short half-time life due to protease degradation, and their sometimes-unwanted toxicity are limiting their clinical applications [1]. A strategy to overcome HDP degradation is the covalent immobilization of the peptide onto the surface of biomaterials, which in turn is expected to endow the biomaterial with bioactivity [2]. Such bioactivity will depend on the peptide density, orientation and conformation on the biomaterial surface, therefore the chemistry used for the covalent immobilization will greatly influence the properties of the HDP-material conjugate [2]. In the present work, we investigated the effect of different conjugation chemistries on the bioactivity of immobilized HDPs. We selected wood-derived cellulose nanofibrils (CNFs) as the biomaterial and KR-12 as the HDP, having in mind the potential application of the CNF-KR12 conjugates to treat chronic wounds. Four different CNF-KR12 materials were prepared applying selective and non-selective conjugation chemistries, and the cytotoxicity and anti-inflammatory properties of the conjugates were evaluated. Molecular dynamics (MD) simulations were performed to obtain an insight into the peptide exposure and conformation when immobilized onto CNFs.

METHODS: KR-12 derivatives were covalently immobilized on CNFs using three different chemical approaches: the non-selective amine coupling through carbodiimide chemistry and the selective thiol-ene click chemistry, and Cu(I)-catalyzed azide-alkyne cycloaddition. RAW 264.7 macrophages were exposed to suspensions of the CNF-KR12 materials and cell metabolic activity and cell membrane integrity were evaluated as indicators of cell viability. To assess the anti-inflammatory effect of the materials, the levels of TNF-α secreted by RAW 264.7 cells simultaneously exposed to lipopolysaccharide (LPS) and KR12-CNF materials were quantified by an ELISA assay. MD simulations were performed to evaluate the accessibility and conformation of the KR-12 derivatives when immobilized onto CNFs.

RESULTS: The results showed that KR-12 endowed CNFs with anti-inflammatory properties and those conjugated by thiol-ene chemistry were the most bioactive materials. MD simulations data showed the most active CNF-KR12 conjugate presented the most accessible peptide, which was also the only one able to display an α-helix secondary structure. Interestingly, some of the conjugates showed cytotoxic effects, while the free peptides and c-CNF were not cytotoxic. This may suggest that the immobilization of the peptides onto CNFs protected them from serum components that normally inhibit their activity.

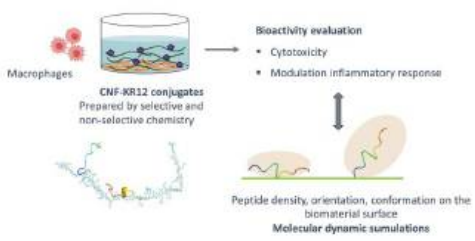

Fig. 1: Schematic representation of the experimental work done in the present study.

DISCUSSION & CONCLUSIONS: KR-12 conjugation via the thiol-ene reaction yielded the most bioactive KR12-CNF composite, a finding than can be attributed to the peptide conformation and accessibility, controlled by the selectivity of the immobilization chemistry and length of the linker in the conjugate. This work provided valuable insights into the effect of functionalization chemistry on the bioactivity of immobilized HDPs.

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Water-based inks for use in extrusion 3D printed instrumented tissues

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INTRODUCTION: Historically, biomedical research has relied heavily on animal studies and traditional cell culture. More recently, microphysiological systems (MSPs) have been presented as an alternative means of studying tissues *in vitro* [1]. MSPs often contain mechanically compliant components capable of conforming to or deforming with biological tissues. Such devices have the potential to produce biological data more efficiently and accurately, particularly when integrated with flexible electronics.

Mechanically compliant components in MSPs have largely been fabricated from polydimethylsiloxane (PDMS) elastomers. However, there is motivation to develop alternative materials for this application because PDMS has limited cell adhesion and tends to adsorb hydrophobic small molecules (e.g. many pharmaceuticals) [2].

Here, I will summarize our work developing latex inks as potential materials for use as ductile components in MSPs. Using a dissolution and dispersion method, latexes of a variety of common ductile polymers can be produced (e.g. polyisobutylene). A polyvinyl alcohol-based surfactant partially functionalized with aryl azide pendant groups is used as a surfactant. This surfactant serves the dual purpose of stabilizing the latex and enabling conjugation to cell adhesive proteins.

METHODS: Inks are formulated using a dissolution and dispersion method enabled by probe sonication. Rotational rheometry is used to characterize the viscoelastic properties of the inks. Demonstrations of printing are done on a direct-ink-writing 3D printer. Bioconjugation is characterized using fourier transform infrared spectrometry and x-ray photoelectron spectroscopy.

RESULTS: I will explain the formulation of these printable latexes (i.e. composition). Next, I will describe the rheological properties of these inks and connect these properties to their ability to be printed using extrusion-based 3D printing. Finally, I will summarize our work on photochemically conjugating these materials to cell adhesive proteins (e.g. porcine gelatin) and outline the implications of this conjugation to cell adhesion and long-term cell culture.

DISCUSSION & CONCLUSIONS: Elastomeric latexes have the potential to be used as ductile cell-adhesive substrates that, through 3D printing, can be fabricated for use in microphysiological systems. Additionally, the addition of photoreactive groups in these latex formulations (in this case arly azides) may be a pathway for conjugating them to cell adhesive proteins. The ability of these materials to reach this potential requires careful consideration of ink composition and processing to ensure they can be fabricated with reasonable resolution.

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ACKNOWLEDGEMENTS: This work is funded by the Novo Nordisk foundation.

Creating soft 3D tissue models for studying ischemia in body-on-chip systems – A research overview of the Centre of Excellence in Body-on-Chip Research (CoEBoC)

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INTRODUCTION: CoEBoC aims to develop body-on-chip (BoC) systems to study ischemia-related pathologies which are leading causes of diseases with high mortality and morbidity rates worldwide. We combine multidisciplinary expertise in human stem cells, biomaterials, sensors, microsystems and biomodelling and -imaging as well as clinical knowledge. The aim is to develop multitissue models for studying tissue interactions when exposed to hypoxic conditions. The focus is on developing brain, heart, liver, and adipose tissue models, all integrated with vascular structures and peripheral tissues with neural connections (innervation). These form the key tissues of ischemic injury and are key players to understand ischemia-related pathophysiologies.

The main biomaterial challenges in these systems are in long-term stability of hydrogels, their capability to support cell growth and behaviour as well as in developing the chip platforms.

METHODS: Biofunctionalized hydrogels withstanding hypoxia are developed to support and control the cell behavior in 3D. Chemical functionalization of hydrogels, including methacrylated gelatin (GelMA) and gellan gum, is done to tune biological and rheological properties. Various differentiation techniques for human-induced pluripotent stem cells and adult stem cells are developed. The oxygen content is regulated and monitored in the BoCs. Several sensing and imaging technologies are integrated into the BoCs including electrophysiology, bioimpedance, and optics-based measurements with the focus in 3D applications. Computational in silico models of cellular functions are developed.

RESULTS: We have formed several tissue and patient models in 2D, organ-on-chip, and 3D formats. Various chemical modification approaches of GelMA have been studied. For instance, gallic acid (GA)-functionalized GelMA with sequential cross-linking using catechol-Fe^{3+} improved hydrogel stability and enhanced viscoelastic properties [1]. Four chemical modifications of gellan gum hydrogel for regulating vascular guidance was developed [7]. Biofunctionalized gelatin-gellan gum hydrogels support vascular network formation [2]. Collagen, fibrin, hyaluronic and gellan gum based hydrogels have been used for creating tissue-specific vascular model [3], neuro-vascular model [4], bone model, neuronal models [5] and vascularised skeletal muscle model. Innervation in cornea-models have been shown with aid of 3D bioprinting [6].

We have also developed acute and chronic hypoxia chips including oxygen sensing. We can gain spatiotemporal control of oxygen with the possibility to change the oxygen content in a few minutes. [7] We have developed human cell-based and electrophysiologically functional cardiac innervation on-a-chip in a compartmentalized microfluidic device [8], Neuronal and vascular 3D models and their computational 3D constructions are under vigorous development [9], [10].

DISCUSSION & CONCLUSIONS: We have developed several significant 3D tissue models and BoC approaches with a focus on studying hypoxia. The future focus is to combine the innervated and vascularised tissue models into more complex multitissue models for studying ischemia in brain, cardiac and adipose tissue and their systemic effects.

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ASTROCARDIA: A vascular heart-on-chip model in space to study cardiac ageing

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INTRODUCTION: Cardiovascular diseases (CVDs) currently represent one of the leading causes of death worldwide. Ageing induces functional changes in the heart that increase the risk of CVDs and impair functional capacity. [1] However, the causes of age-related CVDs are still poorly understood. As of today, no adequate human-derived model exists for studying cardiac ageing.

It has been reported that exposure to the space environment causes accelerated cardiac ageing, leading to the pre-development of several heart conditions induced by radiation- and microgravity-mediated inflammation, DNA damage, and senescence as well as impairment of DNA damage repair pathways. Although vascularized cardiac patches, heart organoids and vascular beds have already been successfully 3D bioprinted here on Earth, progress in the research of 3D bioprinted heart tissue in space is still in its early stages. [2]

In recent years, advances in organ-on-chip technologies have allowed the generation of biological platforms for drug testing and disease modelling that recapitulate human physiology more accurately than traditional 2D cultures and animal models. [3] Furthermore, by applying multiphoton lithography to an organ-on-a-chip platform, macro- and microvasculature can be integrated through addition of endothelial cells and growth factors. In this way, the supply of nutrients and oxygen can be guaranteed. The present research focuses on the development of a human-derived vascularized heart-on-chip model for testing the influence of cardiac ageing in space.

METHODS: Heart-on-chips were bioprinted using cardiomyocyte spheroids as a platform to study cardiac ageing. To this end, human iPSC-derived cardiomyocyte spheroids were encapsulated in a commercial bioink HYDROBIO INX U200 (BIO INX) and printed into microstructures using an UPNANO NanoOne multiphoton lithography system.

RESULTS: After printing, the structures showed high cell viability (Figure 1 a) and maintained the beating over the course of 3 weeks. As proof of concept, hollow channels were designed (Figure 1 b) and printed using HYDROBIO INX U200 (Figure 1 c). Spheroids were oriented in the centre of the hollow channels (Figure 1 d).

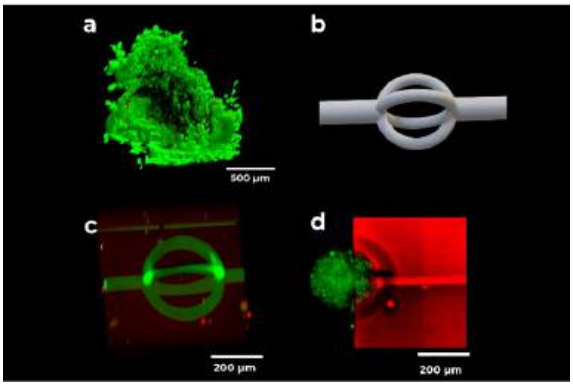

Fig. 1: (a) Fluorescence microscopy image of calcein AM / propidium iodide stained cardiomyocyte spheroids encapsulated in HYDROBIO INX U200 (3 weeks) (b) CAD model of hollow channels (c) Fluorescence microscope image of the channels injected with FITC-dextran (d) a spheroid oriented in the centre of the channels.

DISCUSSION & CONCLUSIONS:

This study is scheduled for a mission to the ISS in 2025. In space, human-derived heart-on-chip models will be exposed to microgravity and space radiation. The resulting changes will be evaluated and compared to ground controls in view of developing a model of cardiac ageing. The development of a new model of cardiac ageing may lead to improved therapeutic strategies for personalised medicine on Earth and in space.

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Exploring Electrohydrodynamics based Functional Nanofibers as Multi-dimensional Nano-Biointerfaces

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The significance of the overall fibrillar and porous nanoscale topography of the extracellular matrix in promoting essential cellular processes has led to consideration of biomaterials with nanofibrous features. Of the many methods for fabricating fibers with micrometer and nanometer diameters, electrohydrodyanmics (EHD) based spinning is simplest, most straightforward and cost-effective. This approach becomes intriguingly powerful when remarkable morphological features were combined with unique chemical, physical, or mechanical functionalisation with ease and control [1, 2].

Alongside the widely studied pathways of biochemical regulation by chemokines, cytokines and growth factors, one often-overlooked but significant influence over the behavior of biological systems is electrical signaling. Voltage gradients among all somatic cells (not just excitable nerve and muscle) control cell behavior, and the ionic coupling of cells into networks via electrochemical synapses allows them to implement tissue-level patterning decisions, which is called developmental bioelectricity. Electrical modulation is therefore a potential target for many new therapies for a range of diseases and biological functions. Our current research focuses on advancing EHD technologies to explore multi-dimensional nano-biointerfaces that synergise the nanostructural induction and the bioelectrical/biochemical signalling to affect cellular behaviours, for biomedical applications in neural and cardiac stimulation and tissue engineering [3-7].

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3 minutes

Oral Presentation

Research of biodegradable nanocomposite based on polyvinyl alcohol and tungsten nanoparticles

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INTRODUCTION: Accumulation of electronic waste and scarcity of raw materials has created major issues for the electronic industry. To address these issues, using biodegradable materials would be an appealing choice. In electronics, a substrate acts as the host on which various functional layers are deposited. Besides, conductors are an integral part of transient electronics. Since the substrate serves intralayer isolation properties to avoid crosstalk, integrating materials from multiple categories is severely constrained by the fact that their compatibility stands as an obstacle. Therefore, it is imperative to develop a new class of biodegradable materials based on polymer-metal hybrids.

Polyvinyl Alcohol (PVA) is considered one of the remarkable biodegradable polymers due to its thermal and chemical resistance, non-toxic nature, affordability, optical qualities, high tensile strength, and excellent biocompatibility [1].

To embed conductivity properties into the polymer structure, metal particles have been used. Biodegradable metals like Magnesium, Zinc, and iron nanoparticles have been explored for creating conductive nanomaterials. However, they display lower conductivity due to the formation of native oxide layers compared to bulk metals. In recent years, the transition metals tungsten (W) and molybdenum (Mo) have garnered more attention as they not only offer good electrical conductivity and can be incorporated into polymer matrix at ambient conditions, but also compared to their metal bioresorbable competitors, they exhibit more gradual dissolving kinetics [2].

This study involved the synthesis of nanocomposite consisting of tungsten nanoparticles (W NPs) and PVA.

METHODS: The PVA powder was dissolved in distilled water to produce the polymer solution. Using vigorous agitation and ultrasonication, different quantities of W NPs were dispersed inside the PVA solutions. Then the solution casting approach was used for making PVA/W nanocomposite film. An investigation was conducted on the impact of W NPs on the structural, optical, and electrical properties of nanocomposite. The films were characterized by X-ray diffraction (XRD), Four-Point Probe, and Ultraviolet–visible (UV-vis) spectrophotometer.

RESULTS: Regarding the XRD pattern, the width of the 2θ peak grows proportionally with the rise in the NP content. Furthermore, the shift to higher angles experiences an increase with NP increment. The UV-vis data demonstrates that nanocomposite samples show a noticeable growth in the absorption coefficient and a decrease in transmittance with the introduction of W NPs. In addition, the results indicate an improvement in electrical conductivity when the concentration of NPs in the nanocomposites increases.

DISCUSSION & CONCLUSIONS: The XRD data reveals that the presence of W NPs greatly enhances the crystallinity disorder of PVA structure and leads to a decrease in the size of the crystallites, ascribing to the dispersion and aggregation of W NPs in the matrix. The increase in absorbance is attributed to the significant absorption of W NPs. Besides, the redshift in the absorption edge of nanocomposites indicates a reduction in the energy band gap. The enhanced disorder in the PVA matrix is explained by the localized state in the bandgap, arising from the role of NPs as electron pathways. The reduction in the transmission spectrum of nanocomposites is assigned to the feature of NPs as scattering centers. The boosted conductivity corresponds to the high electrical conductivity of W metal. The concentration of W NPs within the PVA matrix enhances the amount of weakly disordered, localized electrons, resulting in a highly conductive network.

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Novel Adhesive and 3D-bioprintable Bioinks for Treatment of Corneal Tissue Engineering

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INTRODUCTION: 3D-bioprinting is a game changer in tissue engineering because it allows highly controlled structure preparation such as cornea mimicking structures to deal with global shortage of donor corneas for corneal transplantation and help with millions who need the transplant [1]. The emerged technology evoked a need for new and tailorable 3D-printable bioinks. Non-biocompatibility, weak wet adhesion, poor mechanical strength, need for UV-light or harmful crosslinkers are major challenges of currently available options.

Hyaluronic acid (HA) is found in the native eye and has multiple sites for chemical modifications; it's attractive base material for novel bioinks. We demonstrate chemical modifications for HA-based functional bioinks that have adhesive properties and uses a cell-friendly hydrazone crosslinking to form hydrogels.

METHODS: Hyaluronic acid was modified through EDC/NHS reaction with catechol-derivatives and crosslinking components. Successful modification of HA was confirmed with structural and chemical analysis using ATR-FTIR, ^{1}H and ^{13}C NMR spectroscopy. Degree of modification was determined with both ^{1}H NMR and UV-Vis spectroscopy.

Mechanical characterisation consisted of rheological measurements to evaluate viscosity, shear thinning and storage and loss moduli (G' and G'') of fully formed hydrogels. Compression testing of damaged discs proved self-healing capability and tensile testing for adhesion of synthesised materials.

Printability of bioinks was tested by printing net structures through a 27G needle. Filament thickness and pore factors were used to characterise printability. We used our previously reported bioink [2] as comparison point to see, how introducing new moieties affect mechanical and printability properties.

RESULTS: NMR proved successful modification of hyaluronic acid without compromising the cell-friendly Schiff base crosslinking method. The addition of catechol moieties improved the adhesion of the bioink while allowing good printability. HA-based bioink showed shear thinning behaviour critical for extrusion printing and shape fidelity.

DISCUSSION & CONCLUSIONS: Developed materials fill demands of next generation bioinks with excellent printability, stability and biocompatibility. They are elastic and adhere to surfaces. The hydrazone crosslinking method is dynamic; the structure heals itself after subjected to deforming stress. These adhesive bioinks show promise for tissue engineering applications.

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ACKNOWLEDGEMENTS: We thank Business Finland for providing funding for the research.

Versatile Gallium-based Nanoparticles as Antibacterial Agents

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INTRODUCTION: Antimicrobial resistance (AMR) is recognized as a worldwide public health threat by the WHO. It is estimated that, by 2050, AMR could result in 10 million deaths annually [1]. Therefore, the demand for novel antimicrobial treatments arises from the rising tolerance in bacterial infections and the diminishing effectiveness of current antibiotics [2]. Meanwhile, it is valuable to develop novel antimicrobial strategies that do not rely on traditional antibiotics, such as the targeting of essential nutrient uptake in bacterial pathogens.

Bacteria reply on ferric ions (Fe^{3+}) to sustain their metabolism and enzyme activity. Although gallium ions (Ga^{3+}) share various chemical similarities with Fe^{3+}, such as ionic radius and coordination chemistry, Ga^{3+} cannot undergo reduction under physiological conditions or engage in redox reactions [3]. As a result, the introduction of exogenous Ga^{3+} can disrupt bacterial metabolism and growth.

METHODS: Various sizes and gallium concentrations of gallium-based nanoparticles (GaNPs) were synthesized via flame spray pyrolysis (FSP) and thoroughly characterized to determine their physicochemical properties. The antibacterial capabilities of the flame-made GaNPs were studied using time-kill assays and enumeration of colony-forming units (CFU) to assess the effects of size and gallium content on antibacterial efficacy. Additionally, the loading efficiency of the antibacterial peptide, LL-37, onto the GaNPs was measured through Pierce bicinchoninic acid (BCA) assays.

RESULTS: Six distinct sizes of GaNPs were synthesized through FSP with precise control over flame conditions and precursor concentrations, as confirmed by N_2 adsorption and TEM analysis. The specific surface area results exhibited a correlation with the precursor concentration within the flame. The synthesized GaNPs effectively inhibited the growth of *Pseudomonas aeruginosa*, with a minimum inhibitory concentration (MIC) greater than 31 µg/mL. Moreover, the bactericidal efficacy was influenced by both the size and gallium content of the GaNPs. In addition to their antimicrobial properties, GaNPs can serve as drug carriers for delivering antimicrobial peptides. Consequently, the bactericidal efficacy of the GaNP-LL-37 complex was enhanced.

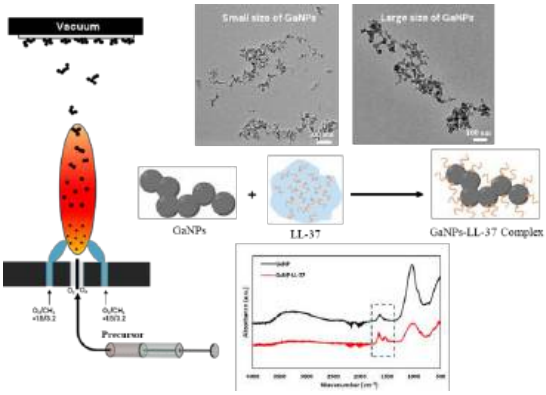

Fig. 1: Schematic representation of flame spray pyrolysis and LL-37 loading onto GaNPs, with TEM images of GaNPs and FTIR spectra of GaNPs and GaNP-LL-37 complex.

DISCUSSION & CONCLUSIONS: In conclusion, GaNPs were successfully synthesized using FSP. These GaNPs display dual functionality, acting not only as effective antibacterial agents but also as versatile nanocarriers for delivering antibacterial peptides. Further experiments will provide the necessary framework for their clinical translation.

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ACKNOWLEDGEMENTS: This project has been supported by the Swedish Research Council (2021-02059).

Supramolecular Self-healing UV-Curable Hydrogels without Photo-initiators using Modified Dextrans

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INTRODUCTION: Supramolecular hydrogels are three-dimensional hydrophilic polymer networks that absorb and retain large amounts of water and are physically cross-linked by supramolecular interactions. The reversible nature of the physical bonds between chains gives the hydrogels viscoelastic properties.[1] In this work, self-healing, shear-thinning, and UV-curable hydrogels were formed by the inclusion complexes between cyclodextrin (CD) host molecules and benzophenone and/or adamantane guest molecules (Fig 1). In detail, the backbones of dextran with different molecular weights were modified with CD as host and benzophenone or adamantane as guest. The inclusion complex formed between CD and benzophenone can lead to the formation of covalent bonds under UV irradiation without the use of photo-initiators, leading to robust hydrogels.[2] Adamantane has a stronger interaction with CD compared to benzophenone, leading to highly viscous solutions. The aim of this study is to assess the physicochemical and biological properties of these novel hydrogel materials for biomedical applications.

METHODS: NMR and FT-IR were used to characterize the chemical structures of the polymers. The self-healing properties were tested by cut-and-heal tests and rheometry. Cytotoxicity tests were performed qualitatively and quantitatively using fibroblasts.

RESULTS: The chemical structure of the modified dextrans was confirmed by the characteristic peaks in the NMR and FT-IR spectra. The hydrogels showed non-Newtonian shear-thinning properties. Depending on the different ratios between the polymers, viscosity varied from 0.91 to 47.8 Pa·s at 37 . Besides, the self-healing property was temperature-dependent, i.e, the higher the temperature, the faster the hydrogel could heal. In vitro assays revealed that the cytotoxicity of the hydrogel was negligible for polymer concentrations below 0.2 g/mL.

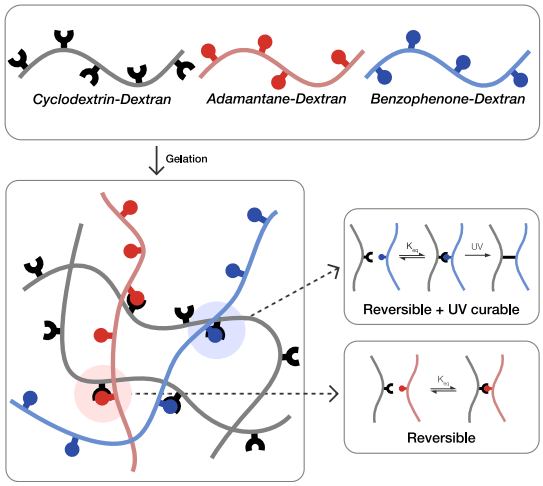

Fig. 1: Graphical representation of hydrogel formation and the reversible and UV-curable supramolecular interactions.

DISCUSSION & CONCLUSIONS: The hydrogel showed shear-thinning and self-healing properties, which could be readily tuned by changing the degree of substitution, the chain length of the dextran backbone, and the solution concentration. These hydrogels represent promising materials for numerous biomedical applications, e.g., as drug carriers and as scaffolds for tissue engineering.

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ACKNOWLEDGEMENTS: This project is supported by a grant from the Independent Research Fund Denmark (nr. 2035-00275B)

Alginate encapsulated bone spheroids: approaches to study bone cells in 3D

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INTRODUCTION: Traumas, fractures and diseases can severely influence bone tissue. A good understanding of the biomineralization process is a fundamental part of developing therapies to enhance bone regeneration. Bone mineralization can be divided into two phases. At first type I collagen is produced by osteoblast. During the second phase, hydroxyapatite crystals will form and precipitate in the gaps between the collagen fibers [1]. The organization of the mineralized ECM is important since it is related to the mechanical properties of bone itself. Given the importance of ECM structure and organization, 2D cell models cannot be applied as standard models to study this process, since in particular they lack cell-ECM interactions, compared to what is found in natural bone. For this reason, the development of a suitable model to study bone mineralization is required. 3D cell systems, in particular spheroids, are a promising approach, due to extensive cell-cell and cell-ECM interactions, and the possibility of combining spheroids with biomaterials [2].

Adequate characterization techniques are required to study these cell models and evaluate not only the properties and characteristics of the mineral produced, but also tissue development process

METHODS: MC3T3-E1 spheroids were obtained using the micro-mold technique [4], and were subsequently embedded into an alginate hydrogel, in which the differentiation, mineralization and the ECM processes were followed. Confocal microscopy was used to study cell organization in the structure. ALP assay, hydroxyproline assay and Second Harmonic Imaging Microscopy (SHIM) will be used to assess cell differentiation and collagen production.

RESULTS: Alginate-encapsulated spheroids were followed in culture for up to 28 days. Confocal microscopy in association with fluorescent dyes and antibodies allowed to visualize the overall structure (Fig. 1 right). Spheroids cultured in differentiation media showed collagen production starting from day 14, observed using SHIM (Fig. 1 left).

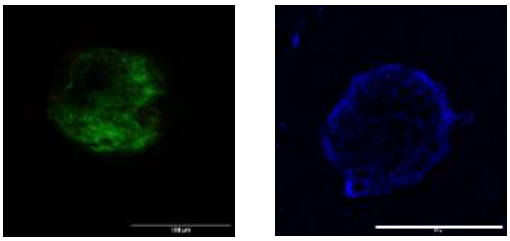

Fig. 1: Spheroids encapsulated into alginate gel and analyzed using confocal microscopy and SHIM. Scale bar: 100 μm

DISCUSSION & CONCLUSIONS: The results show that alginate-encapsulated spheroids can be a suitable model for studying bone ECM deposition and its further mineralization, processes that were not highly investigated in this particular cell system. Electron microscopy can be further used to study collagen and calcium phosphate organization in the spheroids and in the surrounding ECM.

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ACKNOWLEDGEMENTS: We acknowledge Senior Engineer Astrid Bjørkøy and the Center for Advanced Microscopy (CAM) at the Department of Physics, Faculty of Natural Sciences, NTNU for technical assistance and access to confocal microscopy infrastructure. NTNU and NTNU Biotechnology are acknowledged for financial support.

Unlocking Piezoelectric Potential: Terahertz Spectroscopy Characterization of Crystallinity Changes in Flexible and Bioresorbable Poly-L-lactic Acid with Tunable Piezoelectric Properties through Mechanical Stretching

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INTRODUCTION: This study uses Terahertz Time-Domain Spectroscopy (THz-TDS) to characterize mechanically stretched Poly-L-Lactic Acid (PLLA) films for biomedical devices. Distinct changes in absorption spectra, corresponding to various post-treatments, reveal a notable correlation with the degree of stretching. The observed increase in absorption at higher stretching percentages provides a unique avenue for studying crystalline changes induced by mechanical treatments. THz-TDS proves to be a powerful tool, offering insights that traditional vibrational spectroscopic techniques might overlook.[1]

It is generally known that the THz characteristic peak originates from the lattice vibration of the crystalline region. In our study, we attribute the characteristic peak at 2.00 THz to the collective vibration, providing valuable information about the molecular dynamics and structural alterations induced by mechanical stretching.

Building upon the success of post-treatments, our results demonstrate a clear correlation between stretching percentage and absorption intensity, providing a quantitative means to explore crystallinity changes. Specifically, we find that the content of crystallinity increases proportionally with the degree of stretching. This correlation highlights the efficacy of THz-TDS in characterizing the molecular transformations in PLLA films, a capability not easily achievable with other vibrational spectroscopic methods.

This study enhances our understanding of polymer crystallization kinetics. Insights from THz-TDS guide the optimization of PLLA film properties, specifically enhancing piezoelectric responses. These advancements offer promise for developing low-cost, easily deployable biomedical devices, underscoring THz-TDS's significance in biomaterial characterization and design.

METHODS: THz-TDS, conducted with the Toptica TeraFlash system (0.1 to 6 THz range), employed dry air-purged samples. Measurements (1000 averages, 200 µs window) were taken at room temperature, referencing dry air. Samples, affixed to a rotational holder, underwent scans at 0-180 degrees, where 0 degrees aligns with the electric field polarization.

RESULTS: Our investigation into the impact of rotation on the THz peak in PLLA films revealed a consistent trend. For 50%, 75%, and 100% stretched films, the maximum amplitude occurred at 90 degrees, showcasing directional sensitivity (Fig. 1). In contrast, the unprocessed film showed no clear trend, emphasizing the significance of processing.

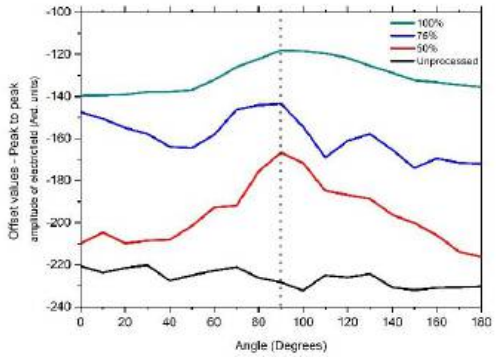

Fig. 1: Rotation Sensitivity of THz Peak in PLLA Films for the unprocessed, 50%, 75% and 100% stretched film

DISCUSSION & CONCLUSIONS: THz-TDS analysis of PLLA films reveals stable absorption in unprocessed films and a distinctive 2 THz peak in processed ones, indicating changes in crystallinity. Notably, increased 2 THz absorption at a 90-degree rotation highlights PLLA's directional sensitivity to the electric field. This insight is crucial for tailoring PLLA films for controlled and directional piezoelectric responses, allowing optimized performance. In conclusion, the 2 THz peak guides the engineering of PLLA films for tunable piezoelectric devices with enhanced application-specific performance.

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ACKNOWLEDGEMENTS: We sincerely thank Pernille Klarskov Pedersen for her generous support, providing access to terahertz equipment and valuable technical assistance, enhancing our project significantly.

Protease-activated delivery of antimicrobial peptides from mesoporous silica

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† Equal contributions.

INTRODUCTION: Antibiotic misuse has led to a dramatic increase in bacteria resistance against all known antibiotics. Novel antimicrobial agents that can combat resistant bacteria are urgently needed. Antimicrobial peptides (AMPs) are an interesting alternative to overcome this worldwide safety problem as they exhibit advantages such as a low propensity for bacteria resistance development, and broad-spectrum activity. However, AMPs are often easily degradable and to some extent cytotoxic causing hemolytic side effects. Therefore, a delivery system providing a protective surrounding and controlled AMP dosage is required. A highly potent AMP (*L*-6-C5) has recently been shown to effectively eradicate infections in clinical isolates with multiple multidrug-resistant bacteria [1]. SBA-15-type mesoporous silica nanoparticles (MSNs) are versatile carriers due to their physicochemical and biocompatible characteristics.
In this work, we investigate the possibility of using bovine serum albumin (BSA) capped SBA-15 as a protease-responsive delivery system of *L*-6-C5 for the treatment of wound infections.

METHODS: SBA-15 MSNs were prepared following the protocol from Björk et al. [2]. The external surface of SBA-15 was functionalized with amino groups [3]. This material is referred to as SBA-15-NH_2. The synthesized lipopeptide *L*-6-C5 was modified with the fluorophore AMCA via an N-terminal deprotonation strategy. SBA-15-NH_2@*L*-6-AMCA was prepared by incubating *L*-6-AMCA with SBA-15-NH_2. The final product was collected and capped with BSA. A peptide release experiment was performed by incubating SBA-15-NH_2@*L*-6-AMCA@BSA in Tris buffer and Trypsin solution (pH = 7.4) at 37 ℃. At each data point, an aliquot was extracted to determine the amount of free *L*-6-AMCA by fluorescence spectroscopy.

RESULTS: The AMP loading efficiency of the capped SBA-15-NH_2@*L*-6-AMCA@BSA and uncapped SBA-15-NH_2@*L*-6-AMCA was 92% and 93%, respectively. The AMP release profile of both delivery systems in the presence of Trypsin as a protease model is shown in Figure 1.

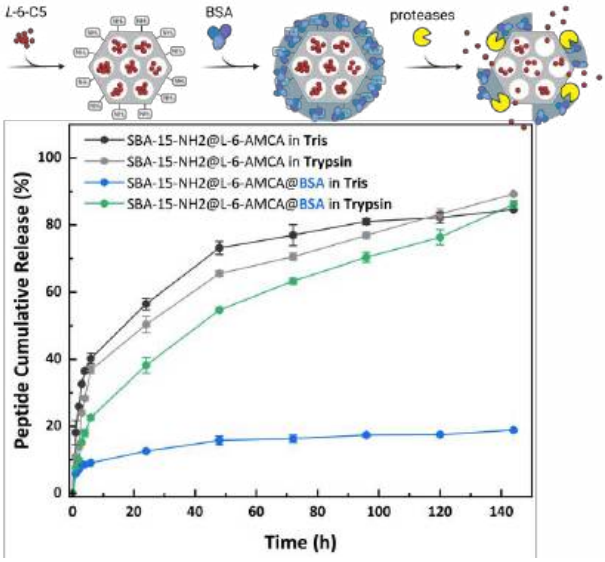

Fig. 1: Cumulative *L*-6-C5 release from MSNs delivery systems in Tris and Trypsin solution.

DISCUSSION & CONCLUSIONS: From the as-prepared MSN delivery systems, 60% of *L*-6-C5 was released after 1 day using uncapped particles, SBA-15-NH_2@*L*-6-AMCA. However, SBA-15-NH_2@*L*-6-AMCA@BSA showed considerable retention of the peptide with less than 20% release after 3 days. This confirms a successful BSA capping of the MSNs. Degradation of the BSA capping in the presence of trypsin was observed with a similar kinetic profile to uncapped particles. In conclusion, our experiments confirm a precisely controlled AMP release achieved by a BSA biocompatible capping system with potential applicability in wound infection treatment.

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ACKNOWLEDGEMENTS: Financial support by the Swedish Government Strategic Research Area in Materials Science on Functional Materials at Linköping University (Faculty Grant SFO-Mat-LiU no. 2009-00971), the Swedish Research Council (VR, grant no 2021-04427), and the Swedish Foundation for Strategic Research (SFF) (HEALiX, grant no RMX18-0039).

Tailored design of macrophage-oriented Metal-Organic Frameworks for robust gene silencing

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INTRODUCTION: Target delivery of small interfering RNAs (siRNAs) to atherosclerotic lesional macrophages has so far remained challenging, represents a research gap for clinical translation. Here, we designed and fabricated a versatile platform that can target to inflamed macrophages and function as RNAi machinery. Following a post-synthetic modification on Metal-Organic Framework (MOF), a polysaccharide phosphate-functionalized MOF nanoparticles (PGMF NPs) showed improved colloidal stability and biocompatibility compared to their parent MOF NPs. The developed PGMF NPs showed inherent peroxide-mimic activity as ROS scavenger. In a preclinical murine model with atherosclerosis, the macrophage-oriented PGMF NPs showed synergetic capability as antioxidant and inflammation resolution vehicle. Altogether, these findings provide proof-of-principle evidence for rational design of macrophage-targeted nanoplatform for gene therapy, which holds great potential for treating atherosclerosis.

METHODS: MOF-based nanoparticles were designed and modified for siRNA delivery. Iron-coordinated MIL-53 MOF NPs was synthesized via hydrothermal method, as previously described.[1] The morphology of nanoparticles was characterized by transmission electron microscopy (TEM, TalosF200i, Thermo Fisher, USA). The antioxidative activity of PGMF NPs was evaluated by DCFDA, followed by flow cytometry analysis to quantify the corresponding fluorescence. The sample preparation procedure was the same as described before.[2]

RESULTS: Here, iron-paired MOF NPs were synthesized, which was further functionalized by polysaccharide phosphate to improve the colloidal stability. The morphology of MIL-53 (Fe) and PGMF NPs were checked by TEM analysis (Fig. 1 a-c). The size of two MOF NPs was identified by DLS assay, both of them showed good dispersity (Fig. 1d). In an LPS-induced inflammatory cell model, the developed PGMF NPs showed inherent ROS scavenging activity compared with control groups, suggesting the peroxidase mimetic activity derived from MIL-53 (Fe).

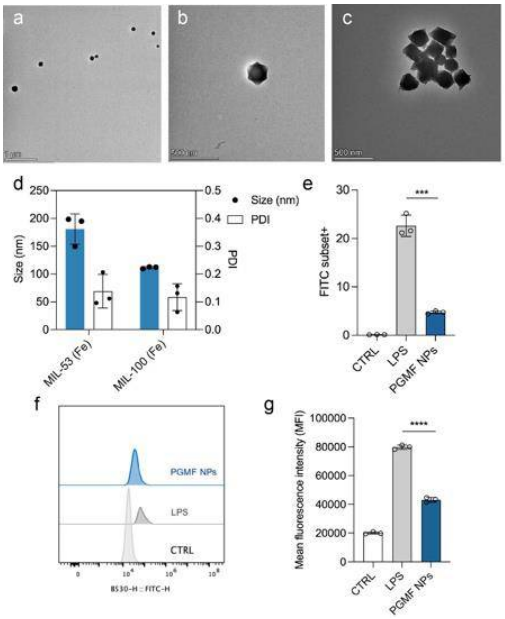

Fig. 1: a-b, Representative TEM images of MIL-53 (Fe) and c, PGMF NPs. d, Size and PDI of MIL-53 (Fe) and MIL-100 (Fe). e-g, Flow cytometry analysis to quantify the fluorescence intensity.

DISCUSSION & CONCLUSIONS: Our findings suggest PGMF NPs as promising optimized nanoplatform for siRNA delivery, further validates the rational design strategy for post-modification of MOF NPs as drug delivery system.

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Can mechanically overloaded implants re-osseointegrate?

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[2] *WMG, University of Warwick, Coventry, United Kingdom*

INTRODUCTION: The success of titanium implants in bone repair and restoration of function is tied to osseointegration, the direct contact between living bone and the implant surface [1]. Mechanical overload can lead to implant failure, especially in cases of low bone volume and/or poor bone quality [2]. It is thought that intentional disruption of bone-implant interface can eventually lead to better implant stability and re-osseointegration [3]. Understanding the biological events triggered by aseptic mechanical failure, as well as the consequences of such trauma on bone microarchitecture and chemical composition, is crucial. Here, we present an *in vivo* model for multiscale and multimodal analyses of the bone-implant interface following mechanical overload.

METHODS: Screw-shaped titanium implants were micro-roughened by alkali etching. Briefly, the implants were exposed to 5M NaOH for 24 h at 60 °C, followed by dry heat treatment at 200 °C for 24 h (Fig. 1A). One implant per tibia was placed into the tibiae of female Sprague Dawley rats. After four weeks of unloaded healing, mechanical overload was simulated in one site per animal by snap-disruption of the bone-implant interface. Surgically exposed implants were turned 90° clockwise, followed by an equal counterclockwise turn returning the threads to the original position. Contralateral implants underwent sham surgery, exposing the implants but leaving them undisrupted. Both the disrupted and undisrupted implants were allowed to heal for a further four weeks. Calcium-binding fluorescent dyes were subcutaneously administered to track bone formation, remodelling, and mineralisation prior to and after snap-disruption. Biomechanical anchorage was evaluated through removal torque measurements at the endpoint (Fig. 1B). Implants with surrounding bone were subjected to X-ray micro-computed tomography (n = 6), dynamic and static histomorphometry (n = 6, Fig. 1C), immunohistochemistry (n = 3), electron microscopy (n = 6), and Raman spectroscopy (n = 6).

RESULTS: At 4 weeks of healing in rats, a period indicative of steady-state bone turnover and established osseointegration, we observed that the disruption of the bone-implant interface did not compromise biomechanical anchorage. Comparable biomechanical stability was measured between disrupted and undisrupted implants, averaging 9.4 ± 2.9 Ncm and 8.7 ± 1.7 Ncm, respectively.

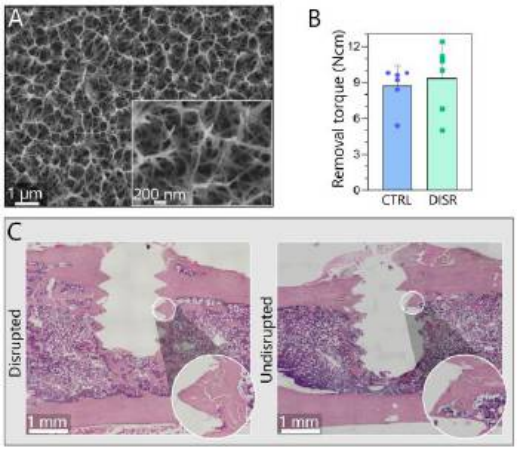

Fig. 1: A) Scanning electron microscopy image of alkali-etched implant surface. B) Biomechanical stability of implants at the endpoint. CTRL = undisrupted, DISR = disrupted implants. C) Representative overviews of haematoxylin and eosin-stained sections of decalcified, paraffin-embedded samples. Insets – new bone formed within the implant threads.

DISCUSSION & CONCLUSIONS: Our findings challenge the conventional notion that disruption of the bone-implant interface exacerbates implant biomechanical stability. It is concluded that, in the absence of compromising systemic conditions, it is possible to achieve re-osseointegration of previously osseointegrated but mechanically overloaded metal implants.

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Aqueous two-phase emulsion-based bioink enhanced by phosphorylated cellulose nanofibers

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INTRODUCTION: Bioprinting is an emerging technology in tissue engineering and regenerative medicine, with the potential of tissue-mimicking constructs composed of living cells and biomaterials for various applications. Bioink development is crucial for creating complex, functional structures and supporting cellular activity in the bioprinting process. Among the diverse bioink systems, aqueous two-phase emulsion (ATPE)--based bioinks composed of photo-crosslinkable GelMA phase and porogen phase, have gained significant interest [1]. These ATPE-based bioinks exhibit cytocompatibility, versatility, and the ability to form interconnected macropores, enhancing mass transfer and cellular activities. However, challenges remain in optimizing their emulsion stability, mechanical properties, and cellular response.

Recent studies have provided approaches that include altering the porogen phase's properties, employing biocompatible nanoparticles for stabilization improving ATPE bioinks' weaknesses without compromising biocompatibility [2-4].

In this study, we have utilized phosphorylated cellulose nanofibers (pCNF) as multifunctional additives to enhance the stability, mechanical performance, and bioactivity of dextran/GelMA-based ATPE bioinks. The incorporation of pCNF offers additional control over the bioinks' rheological properties and promotes mineralization, underpinning these hydrogels as a promising platform for engineering bone tissues with both structural and chemical mimicking.

METHODS: In this work, we prepared the ATPE bioinks by incorporating the pCNF into the GelMA/dextran-based ATPE for extrusion-based bioprinting. Rheological and mechanical characterizations were performed to investigate the effect of pCNF on the inks. MC3T3 E1 cells were used to validate the biocompatibility of the bioprinting process using pCNF-incorporated bioinks.

RESULTS: Rather than the dextran phase, pCNF was found to be more likely to be partitioned into the GelMA phase in our study. Attributed to the network-forming ability of pCNF and the Pickering effect, the stability of dextran/GelMA emulsion was greatly enhanced.

The pCNF network did not increase the flow resistance of the ATPE inks, but enhanced their shear recovery property. The pCNF_ATPE inks were also able to print complex centimeter-scale hydrogels. Meanwhile, pCNF_ATPE hydrogels showed great preformance in energy dissipation during the cyclic compression test.

In addition, we have observed significantly pronounced metabolic activity of the MC3T3 E1 cells, higher cell viability after bioprinting, as well as higher ALP enzyme activity in pCNF_ATPE hydrogels compard to the ATPE hydrogel of dextran/GelMA.

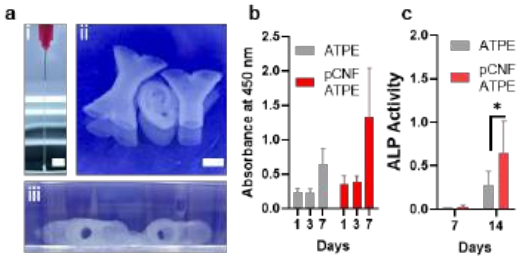

Fig. 1: a) Printability demostration of the pCNF_ATPE inks. Scale bars: 5 mm. b) CCK-8 assay of the MC3T3 E1 cells laden hydrogels during 7 days culture. c) ALP activity of MC3T3 E1 cells in the bioprinted hydrogels on Day 7 and 14.

DISCUSSION & CONCLUSIONS: In this study, we demonstrated that with addition of small amount of pCNF could improve the physical performance and the biocompatability of ATPE bioinks.

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ACKNOWLEDGEMENTS: The authers would like to thank the Research Council of Finland (333158) for their fund in this project at Åbo Akademi University.

Biofabrication of Granular Hydrogel-based Bioinks for Dermal Regeneration

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INTRODUCTION: Wounds imbue a tremendous burden on the health care system and causes vast pain and suffering [1]. Treatment using split-thickness skin grafts results in poor functional and aesthetic outcome, while cultured epidermal autografts fail to recapitulate the full diversity of cells and extracellular matrix components (ECM). We have previously shown that porous gelatin microcarriers (PGMs) can serve as a transplantation vehicle for cells in the treatment of wounds [2]. Here, we investigate cell-laden PGMs in combination with 3D bioprinting to biofabricate tissue-engineered skin for transplantation.

METHODS: Using bioorthagonal copper-free click chemistry, PGMs were functionalized to enable polyethylene glycol (PEG)-azide bridged crosslinking with hyaluronic acid (HA). Human dermal fibroblasts were seeded on the PGMs for three days to ensure proper attachment and expansion. Zombie Red™, immunofluorescent staining, and semiquantitative real-time PCR were used to investigate cell viability and phenotype, as well as ECM production over time. Constructs composed of cell-laden PGMs, HA and gelatin were created using a Cellink Bio X bioprinter, and transplanted to a human *ex vivo* wound model [3].

RESULTS: Fibroblast viability on PGMs was 86.6 ± 5.3 % before printing. Immunofluorescent staining and complementary gene expression assays revealed ECM production on the PGMs, with significant increases of collagen III and Laminin V. The presence of α -smooth muscle actin positive cells on the PGM was confirmed by a ten-fold increase of ACTA2 gene expression.

Directly after printing, viability was 78.9 ± 6.0 %, and 84.9 ± 5.0 %, 75.6 ± 15.9 % after 24 and 72h, respectively. Ki67-positive staining revealed a subset of cells to be in a proliferative state. Cell proliferation was further confirmed by quantification of DAPI-stained nuclei, where the number of cells in constructs increased over time. Proliferative cells were also identified in constructs transplanted to *ex vivo* wounds.

DISCUSSION & CONCLUSIONS: The combination of functionalized PGMs and hyaluronic acid (HA) enabled 3D-bioprinting of tissue constructs. Cells were shown to survive and proliferate over time and produce ECM molecules relevant for dermal regeneration. Current studies focus on *in vivo* evaluation of the tissue constructs using a mouse model.

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Bioactive, tough, and electroactive hydrogels for skeletal muscle tissue engineering

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INTRODUCTION: Skeletal muscle tissue, despite its remarkable flexibility and elasticity, is susceptible to injury due to factors such as overexertion, trauma, lack of warm-up, muscle imbalances, insufficient flexibility, age-related changes, and dehydration, leading to movement impairments and functional deficits. To this end, skeletal muscle tissue engineering (SMTE) has emerged as a promising therapeutic approach for repairing various muscle defects. However, designing scaffolds that can effectively mimic the native properties of skeletal muscle tissue remains a significant challenge [1,2]. Ideally, these scaffolds should be biocompatible, mechanically robust, and capable of promoting cell adhesion, proliferation, and differentiation.
To address these challenges, we present a novel hydrogel based on poly (vinyl alcohol) (PVA), gelatine, and graphene oxide (GO) nanoflakes. This novel hydrogel exhibits intriguing mechanical and electrical properties, making it a promising candidate for SMTE applications. We found out that Incorporating GO nanoflakes into the PVA-gelatine hydrogel significantly enhances mechanical strength, with a threefold increase in elastic modulus and ultimate stress. This improvement is attributed to strong interfacial interactions between GO and the polymer matrix. The hydrogel also exhibits strain-sensitive electrical conductivity, making it promising for bioelectronics. Additionally, it demonstrates excellent biocompatibility, supporting its potential for applications in SMTE.

METHODS: We used PVA and gelatine in an 80:20 ratio, to which we added graphene oxide (1wt% or 2wt%); hydrochloric acid was used as a catalyst and glutaraldehyde was used as a crosslinker. The obtained hydrogels were then investigated chemically (FT-IR, DSC, TGA), mechanically (compression, tension, rheology), electrically (electrochemical impedance spec-troscopy, motion sensing, resistance vs strain) and biologically (cytotoxicity, live/dead). Additionally, bioprinting, weaving and shape-memory capabilities were also explored.

RESULTS: The hydrogel developed presents a wide range of exciting properties, including promising mechanical behaviour, low hysteresis, ionic conductivity, good cell compatibility, motion sensing capabilities, high flexibility, and the possibility of bioprinting it.

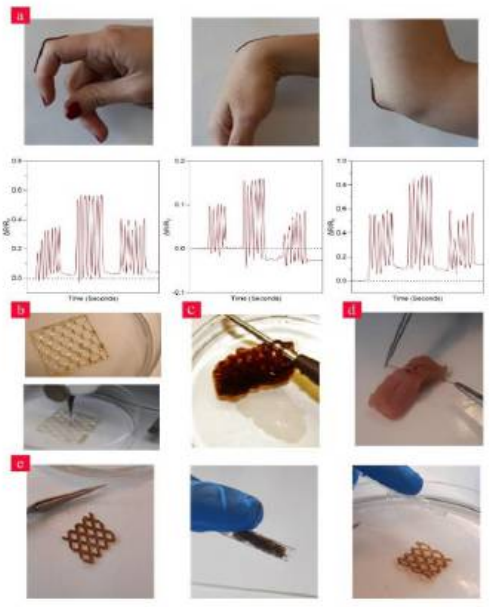

Fig. 1: a) Motion sensing capabilities b) Bioprinting c) Weaving d) Using a 2wt% GO hydrogel filament as a suturing thread e) Memory-shape like behaviour.

DISCUSSION & CONCLUSIONS: The commendable mechanical properties, coupled with its memory-shape behaviour, ability to sense human motion across a wide range of movements, and excellent biocompatibility, position this innovative hydrogel as a highly prospective candidate for minimally invasive applications in SMTE and advancing the field of bioelectronic tissues.

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Cardiac patch of a novel self-healing hydrogel for mending post-infarction defects

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INTRODUCTION: This study focuses on a novel self-healing hydrogel for addressing the damaged tissue and promoting post-infarction healing, by attaching it as a cardiac patch. The specific goals of this research are to characterize the self-healing and mechanical properties of the biomaterial, determine cell viability and differentiation of cardiomyocytes via in vitro studies, and test the bioactivity of the biomaterial through in vivo murine models.

METHODS: The manufacturing of this material implicates a process of chemical functionalization of two natural polymers [1, 2] to form a double network self-healable gel. Material characterization was conducted through Fourier-transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (^{1}H NMR), thermogravimetric analysis (TGA), mechanical tests, rheology, swelling and degradation assessments, and scanning electron microscopy (SEM). *In vitro* studies confirmed the biocompatibility of our hydrogel and provided a detailed recognition of its interaction at a cellular level, laying the foundation for *in vivo* studies to understand the performance, safety, and therapeutic potential of the cell-seeded patch in a live animal model.

RESULTS: FTIR and NMR spectroscopy confirmed the successful formation of the functionalized polymers and their influence on the novel hydrogel. Mechanical compression tests revealed varying compressive moduli, suggesting different combinations of our hydrogel according to the desired clinical application. Swelling studies confirmed this correlation, emerging one of the combinations as a long-term candidate. TGA demonstrated the thermal stability of the combinations and, in the rheology assessment, oscillatory frequency sweep experiments determined hydrogel's mechanical strength and suitability for 3D printing. The hydrogel exhibited self-healing behaviour and viscosity suitable for extrusion-based 3D printing applications. In vitro tests validated that our material is compatible with both cell seeding on the surface of the hydrogel and through encapsulation. After 7 days of seeding cardiomyocytes, immunofluorescence microscopy confirms the development of actin filaments typical of the structural organization of functional muscle. *In vivo* studies are currently being carried out to provide valuable insights for potential future clinical applications in cardiac tissue engineering and regenerative medicine.

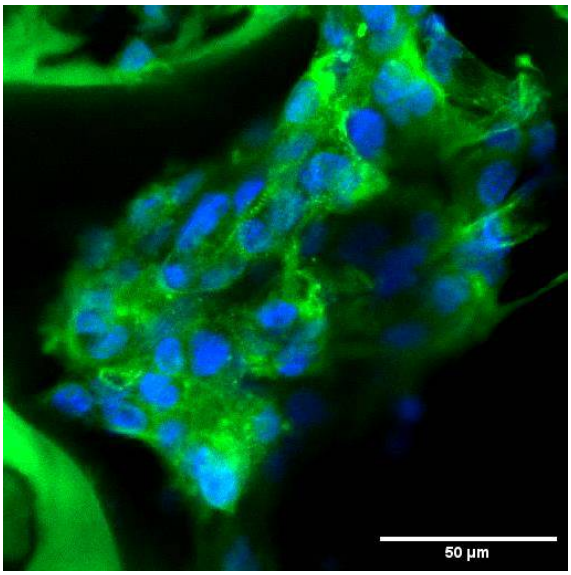

Fig. 1: Immunostaining of cardiomyocytes growing over C3 hydrogels at day 7, showing the actinin filaments compatible with the structure of functional muscle tissue

DISCUSSION & CONCLUSIONS: The proposed cardiac patch, a hydrogel made from natural polymers, demonstrates promising self-healing properties and mechanical strength. Furthermore, the biocompatibility confirmed in *in vitro* 2D studies provides a strong foundation for advancing this biomaterial towards practical cardiac tissue engineering applications.

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ACKNOWLEDGEMENTS: I would like to express my sincere gratitude to the Technical University of Denmark, the Netherlands Organization for Scientific Research (NWO), and the Centre for Applied Medical Research (CIMA)-Universidad de Navarra for their generous funding.

Poster Presentation

Hybrid Phenol-Functionalized Nanoparticles as Efficient Scavengers for Reactive Oxygen Species with Intrinsic Anti-inflammatory Activity

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INTRODUCTION: Reactive oxidative stress (ROS) plays a significant role in various inflammatory diseases, including arthritis, inflammatory bowel disease, and diabetes. Among the ROS-scavenging therapeutics, natural phenols have demonstrated excellent antioxidant properties. Unfortunately, their low water solubility, rapid clearance from the blood, and poor chemical stability hinder their clinical translation. In this direction, recent progress in the synthesis and functionalization of nanoparticles has led to the development of drug delivery systems that can safely and accurately target inflammation. Here, we present hybrid-phenol nanoparticles (HPNPs) for ROS scavenging. The HPNPs were synthesized by one-step polymerization or functionalizing the organic molecules onto inorganic amorphous SiO_2 nanoparticles.

METHODS: DLS and SEM images confirmed the successful nanoparticle synthesis, followed by decreased hydrodynamic diameter and polydispersity in HPNPs compared to the free monomers suspended in water. Dynamic Fourier-transform infrared spectroscopy (FTIR) and Raman were used to identify the antioxidant OH groups on the surface of the HPNPs. *In vitro*, staining with DCFH-DA assay shows the successful ROS scavenging activity of HPNPs.

RESULTS: After synthesizing, the HPNPs improved natural phenols' aqueous solubility and long-term stability. Moreover, FTIR and Raman analysis proved the presence of the antioxidant OH-groups on the surface of the NPs. The radical scavenging activity of HPNPs is evaluated by quenching the free radical DPPH, significantly improving antioxidation activity compared to free molecules. Next, overall toxicity was analyzed using Alamar blue assay in human embryonic kidney (HEK) cells, showing minimal toxicity toward normal cells. Finally, the HPNPs antioxidant effect was further validated *in vitro* after rescuing HELA cells from H_2O_2 oxidation and hypoxia conditions.

DISCUSSION & CONCLUSIONS: Oxidative stress results from an imbalance between ROS production and their elimination by protective mechanisms, leading to chronic inflammation. Natural phenols have a potential anti-inflammatory effect, associated with antioxidant activity and inhibiting enzymes in producing eicosanoids. However, their limited solubility in water and poor chemical stability hinders clinical translation. Hence, developing nanomaterials with efficient ROS scavenging ability, stability, and biocompatibility could be a potential strategy to counteract inflammation. Here, we present a synthesis of polyphenol-containing nanoparticles for depleting ROS. We selected representatives of the most common natural-derived polyphenols, such as stilbenoids (curcumin and resveratrol) and flavonoids (quercetin and gallic acid), to synthesize HPNPs. These findings lay the groundwork for using natural-derived polyphenol nanoparticles for ROS scavenging and demonstrate their potential use in clinical applications.

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Interactions of antimicrobial peptide with antibiotics and the use of amphiphilic hydrogel microparticles for enhanced antibacterial activity

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INTRODUCTION:

The rise of antimicrobial resistance (AMR) and the spread of multidrug-resistant infections have led to combinatory therapies gaining significant interest. Combination of conventional antibiotics (AB) with antimicrobial peptides (AMP) has been deemed a promising approach for prevention and reversal of antibiotic resistance, despite the inherently low stability of AMPs [1]. To improve AMP stability with retained antibacterial activity, covalent attachment onto biomaterials has been extensively reported in literature [2, 3].

Here a novel strategy to enhance and synergize the activity of conventional AB by combining them with AMP functionalized hydrogel microparticles has been explored. Oxacillin (OXA) and vancomycin (VCM) ABs have been combined with plain AMP and AMP functionalized hydrogel microparticles against *Staph. aureus* and methicillin-resistant *Staph. aureus* (MRSA). By synergistic interaction between AMP particles and AB the bacterial antibiotic sensitivity and efficacy has been improved with a potential for repurposing antibiotics and minimizing AMR.

METHODS: AMP (RRP9W4N) functionalized hydrogel microparticles were produced from diacrylated Pluronic F127 based on a previously reported method [3]. Minimal inhibitory conc. (MIC) of AMP, AMP particles, VCM and OXA alone were determined against *Staph. aureus* and MRSA. Checkerboard assays were used to evaluate the potential synergism between plain AMP with OXA and VCM, respectively. Fractional inhibitory conc. (FIC) index was used to categorize the AMP-AB interaction type. Modified checkerboard assay and time-kill studies were employed to evaluate AMP particle interaction with OXA against MRSA.

RESULTS: By combination of plain AMP with OXA the MIC of OXA was reduced from 0.25 µg/ml to 0.13 µg/ml against *Staph. aureus* and from 32 µg/ml to 0.06 µg/ml against MRSA, respectively. MIC of VCM was reduced from 1.00 µg/ml to 0.03 µg/ml against *Staph. aureus* and from 2 µg/ml to 0.06 µg/ml against MRSA, respectively, upon combination with the AMP.

The checkerboard assays of plain AMP with OXA against MRSA displayed most promising results with mean FIC index of 0.39 indicative of synergism. Upon combination of OXA with AMP functionalized hydrogel particles, OXA MIC was reduced by 64-folds from 32 µg/ml to 0.5 µg/ml against MRSA (Fig.1).

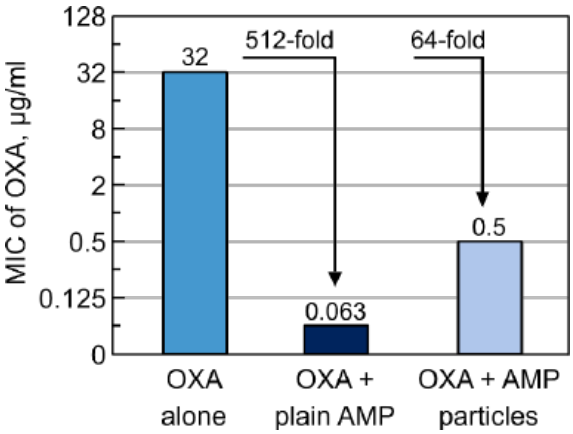

Fig. 1: MIC of oxacillin antibiotic alone and in combination with plain AMP or AMP functionalized hydrogel microparticles against MRSA.

DISCUSSION & CONCLUSIONS: Plain AMPs have been known to exhibit synergism with conventional AB, however no AMP functionalized biomaterials are known to demonstrated synergistic interactions with AB. In this work synergistic interaction with AMP and OXA antibiotic has been demonstrated *in vitro* against OXA-resistant MRSA, both in free AMP state and with AMP functionalized hydrogel microparticles. AMP hydrogel particles show potential applicability in AMP-antibiotic combinatory therapy for repurposing of AB against drug-resistant infections.

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Silk-Based Hydrogels: Versatile Matrices for Biomedical Applications

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INTRODUCTION: Silk, a natural protein fibre renowned for its durability and biocompatibility, has shown promising results for biomedical applications [1]. Recent studies have focused on integrating silk fibroin (SF) into hydrogels - three-dimensional polymer networks known for their biomedical versatility [2]. Mixing chitosan (CS), gelatine (G), hyaluronic acid (HA) and fucoidan (FU) with SF during hydrogel preparation process presents a promising strategy in biomaterial research, enhancing mechanical strength, biocompatibility, and bioactivity [3]. On the other hand, calcium phosphates (CaP) and SF hydrogels show potential in mimicking natural tissues, fostering cellular interactions, and advancing innovative biomedical materials [4]. This study examines the development, characterization, and possible use of silk fibroin - based hydrogels, emphasizing their potential in tissue engineering.

METHODS:

A. Preparation of solutions. SF solution was prepared by boiling *Bombyx mori* cocoons according to the method described before [5]. CS solution was prepared by dissolving CS in 1% acetic acid. FU, G and HA solutions were prepared by dissolving them in water in various concentrations.

B. CaP in situ synthesis in SF. CaO was added in a 10% SF solution with stirring, 2M H_3PO_4 was added dropwise and stirred at 300 rpm to reach pH 6, 8, 10, and 11, obtained slurry was used for further hydrogel preparation.

C. Preparation of hydrogels. Hydrogels were prepared using 3 different methods with various rations between the polymers: 1. Physical crosslinking by freezing (-20 °C / -86 °C) or heating (37 °C / 60 °C) [5]. 2. Enzymatical crosslinking with horseradish peroxidase (HRP) and H_2O_2 [6]. 3. Chemical crosslinking with glutaraldehyde (GTA) [3]. See table below for hydrogel compositions.

Method	Composition
1	CS/FU, CS/SF, FU/SF, SF
2	SF/G, SF/G/CaP
3	SF/HA, SF

Table 1. Compositions of prepared hydrogels

All synthesized samples were characterized with Fourier-transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), swelling, gel fraction, degradation, phase composition with X-Ray diffraction analysis (XRD) and *in vitro* cell viability on mouse fibroblast 3T3 cells.

RESULTS: SF solution treated at higher temperature (60°C) cross-linked faster than solution kept at 37 °C. CS/FU, CS/SF and FU/SF hydrogels frozen at -86°C has smaller pores than hydrogels frozen at -20°C. Physically cross-linked (method 1) SF/FU hydrogels dissolve immediately at all polymer ratios, however SF/CS hydrogels are stable for more than 24h. Enzymatically cross-linked hydrogels containing CaP have a better swelling degree and gel fraction. Chemical crosslinking of SF with HA using GTA proved to be successful and ensured hydrogel stability for more than week. The obtained results show that developed SF based hydrogel samples are not cytotoxic.

DISCUSSION & CONCLUSIONS: Freezing temperature of hydrogels affects their porous structure, heating accelerates the cross-linking time of hydrogels. Addition of CaP to enzymatically cross-linked hydrogels, stabilizes the hydrogel network at pH values above pH 6. Chemical cross-linking requires a minimum concentration of 8% HA and 3.23% GTA.

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Sintering Temperature and Liquid Phase Molarity Influence on Doxorubicin-Loaded Calcium Phosphate Cements: A Comprehensive Study for Prolonged Drug Release in Bone Cancer Therapy

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INTRODUCTION: Osteosarcoma, a widespread metastatic affliction, affects people of all ages. The therapeutic interventions that bone cancer patients undergo are traditionally either surgery to remove the tumor, potentially followed by implant fixation (reconstructive surgery), or chemotherapy, and radiotherapy[1,2]. These conventional methods, however, entail challenges such as cancer recurrence, possible implant failure and inflammation, as well as systemic toxicity, particularly with oral or intravenous chemotherapeutics[3]. Additionally, even if the implant leads to a successful bone regeneration, a second surgery is required for implant removal with further potential risks. Calcium phosphate bone cements (CPCs) are promising biomaterials for bone tissue regeneration and cancer treatment, as they are biocompatible, moldable, injectable, and self-setting. Simultaneously, they are biodegradable and porous, eliminating the need for a secondary surgery and facilitating their loading with chemotherapeutic drugs[4]. In this work, we focus on α-tricalcium phosphate (α-TCP) cements laden with doxorubicin (DOX) drug, exploring the influence of the cement powder sintering temperature and of the liquid phase molarity on the DOX-CPC properties.

METHODS: CPC powder underwent BET analysis for specific surface area, utilizing the Quadrasorb SI gas sorption system after 24h degassing with the Autosorb Degasser Model AD-9. Setting time was determined through the Vicat needle method, and XRD elucidated crystallinity and phase composition. Compressive strength was gauged using an INSTRON 10 kN apparatus, and SEM delineated CPC morphology. DOX release kinetics were examined in a 3.5 ml PBS solution for 9 months, with quantification via UV-VIS spectroscopy ($\lambda = 480$ nm).

RESULTS: Higher sintering temperatures reduced CaP particle surface area, decreasing reactivity and prolonging the transformation from α-TCP to HAp. DOX entrapment decreased setting time at 650°C and 700°C, while higher sintering temperatures extended it by up to 61% for a 100°C increase. Reduced liquid phase molarity delayed setting time, with increases of 73.75%, 58.62%, and 137.73% in time at 650°C, 700°C, and 750°C, respectively. Compressive strength results displayed a 550% increase to doubled molarity. Both liquid phase molarity and powder phase sintering temperature significantly impacted drug release profiles.

DISCUSSION & CONCLUSIONS: Our results underscore the intricate interplay of powder phase sintering temperature and liquid phase molarity, influencing properties such as powder surface area, DOX-CPC setting time, mechanical properties, and drug release, as well as the transformation kinetics to HAp. While DOX-CPC combinations display prolonged drug release over 9 months, a comprehensive in vitro biocompatibility assessment is also required and planned for future experimental studies.

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Metallic Nanoparticles and Their Impact on Dopamine Electro-Sensing

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INTRODUCTION: Alzheimer's and Parkinson's diseases, as progressive neurological conditions, present a pressing need for fast, accurate, and reliable detection methods [1-3]. This project focuses on developing an innovative electrochemical (EC) sensor, by modifying carbon-based materials, for the sensitive and selective detection of dopamine (DA) - a key neurotransmitter implicated in these diseases. Despite the growing use of EC methods for dopamine detection, challenges such as low analyte concentration, fouling (both EC and biological), and host response limit their efficacy. This research aims to overcome these issues by integrating novel metallic nanoparticles (gold and platinum) with carbon-based materials, enhancing the sensor's EC sensitivity and selectivity. The study also explores the impact of nanoparticles' aspect ratios, shapes, and sizes on EC responses to optimize sensing conditions.

METHODS: This research's key method components include A. Chemical Fabrication and Integration of Nanoparticles: A 4-step chemical process is used to fabricate metallic core-shell nanoparticles. These are then integrated onto the surface of carbon-based materials to enhance the electrodes' electrochemical response to DA. B. Chemical and electrochemical Characterization: Using Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM), the nanoparticles are characterized to confirm their structure and geometry, which is crucial for their function in the sensor. Techniques such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV) are employed to characterize and determine DA behavior at low physiological concentrations. C. Addressing Interferences: The study also aims to resolve issues related to interferences (like ascorbic acid and uric acid).

RESULTS: Initial findings are encouraging, indicating that the use of the fabricated nanoparticles enhances the sensitivity for dopamine on a carbon substrate, as shown in Figure 1.a. Furthermore, interference studies conducted with the modified electrode have yielded positive results. Traditionally, the electrochemical detection of DA in the brain is complicated by the overlapping oxidation potentials of uric acid and ascorbic acid, which are prevalent in the brain, with that of DA. This overlap often leads to merged peak currents and inaccurate measurements. However, the recent modifications to our carbon substrates, incorporating fabricated nanoparticles (NPs), have resulted in a platform that demonstrates negligible sensitivity to these interfering substances and a significantly increased affinity for DA (Figure 1.b).

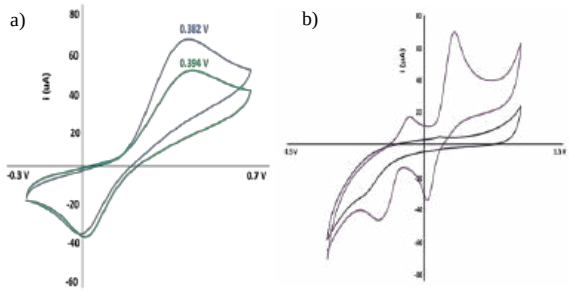

Figure.1 (a) Electrochemical performance of unmodified (green) and modified carbon-based material with Aquept NPs (blue) in a 1mM DA solution. (b) selectivity of Aquept nanoparticles modification. 1mM of ascorbic acid (purple) versus the response for 1mM of ascorbic acid + 1mM DA (pink) in PBS buffer vs. Ag/AgCl reference electrode.

DISCUSSION & CONCLUSIONS: Although these preliminary results are promising, our research will continue by testing different morphologies of Au@Pt core-shells and even move on to test several other types of novel nanoparticles, such as bone-shaped silver nanoparticles. We hope we can provide a comprehensive insight to incorporating these modifiers and what they do to impact the biological selectivity, fouling, and the temporal resolution.

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Biopolymers from Riboflavin for CO_2 capture and O_2 reduction

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INTRODUCTION: Biomaterials are an increasingly important field of research in times of global warming. Due to this omnipresent topic of climate change, efforts are being made to make industrial processes more eco-climate-friendly and to reduce the greenhouse gas emissions. As a result, biomaterials are gaining more and more attention as an environmentally friendly alternative, even if they are not yet completely climate neutral [1]. Examples for promising organic molecules for the reduction of greenhouse gases and the dependency of carbon-based fuels are the naturally occurring molecule classes of anthraquinones and riboflavin (vitamin B2), which are known as efficient, organic photo-electrocatalysts [2]. Both the monomers as well as the respective polymers are reported here for O_2 reduction to H_2O_2, and CO_2 capture and conversion. Furthermore, the monomers can be used in medical applications and in organic batteries, while the polymers can additionally also be used in sensors or electrodes for various devices [3,4]. In this contribution, we report on the successful polymerization and immobilization of two aminoanthraquinones and riboflavin on different carbon-based electrodes using an oxidative electropolymerization process [5].

METHODS: The polymerization of the mentioned biomaterials was performed on different carbon-based electrodes as well as on an optically transparent electrode with an oxidative polymerization in acidic media. The resulting polymers were further characterized using infrared (IR) spectroscopy, cyclic voltammetry (CV) in aqueous media, and scanning electron microscopy (SEM) images, providing insights into the structure-property relations.

RESULTS: In this work we report the electropolymerization and the capability of electrochemical carbon dioxide capture as well as oxygen reduction of these materials. The CV provided further information about the redox properties of the resulting polymers and about the stability as well as the interaction with O_2 and CO_2. The performed IR spectroscopy gave information about the structure of the polymers even though the polymerization of riboflavin onto an optically transparent electrode was not straightforward. SEM images provide a suitable proof of the successful polymerization of these biomaterials.

DISCUSSION & CONCLUSIONS: In conclusion, the polymerization was successfully optimized, and the resulting biopolymers exhibit a homogeneous film and good CV stability on the electrode. The behavior of the polymers under O_2 and CO_2 can be compared with the previous studies of the corresponding monomers [6,7,8]. The IR spectra confirm the successful polymerization and provide information on where the polymerization takes place. In addition, the broadening of the peaks indicates that a conducting/semiconducting polymer is obtained with extended π-electron delocalization.

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QCM-D-based analysis of cell adhesion: a relationship between QCM-D signal change and focal adhesion kinase activation

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INTRODUCTION: Cell interaction with a surface of biomaterials is one of the key factors determining the fate of a biomaterial within a body. An acoustic quartz crystal microbalance with dissipation monitoring (QCM-D) technique allows analyzing the kinetics of interactions between cells and an underlying substrate non-invasively and in real time. QCM-D signals have been proposed to quantitively reflect the dynamics of receptor-mediated cell attachments or establishment of focal adhesion complexes (FAs) through which cells bind to extracellular matrix [1]. The association of QCM-D signals with integrin-RGD binding and their correlation with integrin expression levels have been later proven using photo-activatable RGD peptides [2]. It has been also demonstrated that the areal density of FAs, measured from fluorescence images, correlates with a magnitude of the ΔD response [3]. Here, we aimed to further investigate the relationship between QCM-D signals and FAs establishment. For this, we measured the adhesion of human gingival fibroblasts (hGF) using QCM-D and correlated the obtained responses with activation of focal adhesion kinase (FAK) determined via ELISA as FAK activation has been shown to be an important regulator of assembly and turnover of FAs [4].

METHODS: Cell adhesion process was studied on two substrates: polystyrene oxidized by UV-ozone treatment for 15 min (PS_{ox}) and tannic acid nanocoatings deposited on polystyrene (PS-TA) at pH 7.8 during 1 hour [5]. Cell attachment was monitored in a QCM-D QSense® window module (QWM 401) using a QCM-D QSense® E4 (QSX 310, Biolin Scientific). The sensors (QSX 305) were prepared according to the manufacturer's protocol, oxidized or coated with tannic acid nanolayer, equilibrated at 37ºC in serum-free medium for 1 hour and after that in a respective test medium (without FBS or with 10% FBS) at 50 µl/min for approximately 1 hour. HGFs were then injected into a QCM module at 300 µl/min (30 sec) at a concentration of 0.5×10^6 cells/ml. Measurements were performed at 37ºC under 10 µl/min flow of test medium overnight. Level of FAK activation was determined by using human FAK[pY397] and FAK total ELISA kits (Invitrogen) and confirmed by a immunocytochemistry analysis at 1, 2, 4, 6 and 24 hours of hGFs cultivation in static conditions.

RESULTS: The QCM-D results and microscopic analysis of cells revealed that cell adhesion to the substrates was rapid in serum-free medium but significantly delayed in 10% FBS. Cells adhered to a higher degree to PS_{ox} than PS-TA in serum-free medium. In presence of 10% FBS, hGFs showed slow adhesion to PS_{ox}, whereas cell attachment to PS-TA was almost completely hindered. A strong positive correlation was found between activated FAK and ΔD, but not Δf, for all tested conditions (Fig.1).

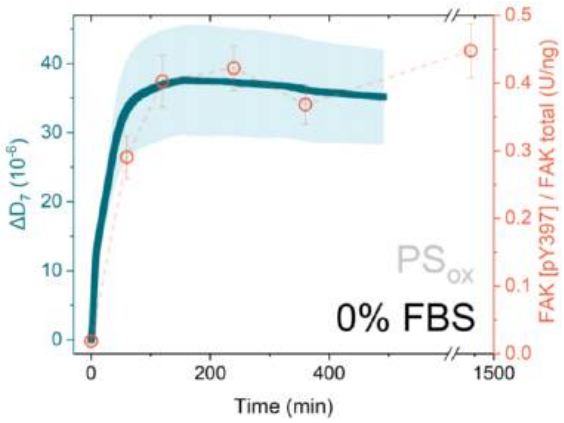

Fig. 1: Fibroblasts adhesion to PS_{ox} surface in serum-free medium. Left axis (blue): change in dissipation (n=7) monitored with a QCM-D. Right axis (red): a degree of FAK activation measured with ELISA.

DISCUSSION & CONCLUSIONS: This study shows a linear correlation between the activation of FAK and QCM-D responses. This result is in agreement with previous studies and confirms that QCM-D is a valuable technique for analysis of early cell adhesion process and establishment of FAs.

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Nanozyme-Mediated Antioxidant Protection of Hemoglobin-Based Oxygen Carriers

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INTRODUCTION: The constant supply of oxygen (O_2) into the tissues of the body is a crucial function for survival. Blood facilitates the delivery of O_2 and in severe accidents or blood diseases, transfusion is the sole method for substitution. Even though transfusion of donor blood is a well-established procedure, it is characterized by some limitations, because of its short shelf-life and the need for blood typing and matching [1]. The development of hemoglobin (Hb)-based O_2 carriers (HBOCs), as blood substitutes is considered a promising alternative with several advantages over conventional blood transfusions.
However, a crucial limitation of current HBOCs is the autoxidation of Hb into metHb, which lacks O_2-carrying capacity [2]. Native red blood cells possess a complex antioxidant system, including catalase (CAT) or superoxide dismutase (SOD) enzymes, able to prevent and revert the conversion of Hb into metHb.
However, the direct incorporation of HBOCs with natural enzymes is limited due to the high production cost, the low stability, and the short catalytic half-lives [3]. A new approach, which includes the use of nanoparticles (NPs) with enzymatic-like activity, referred to as nanozymes (NZs), has emerged as a novel antioxidant system, protecting the Hb from autoxidation into metHb [4].

METHODS: Metal-organic framework (MOF)-based NPs, encapsulating Hb (Hb@MOF NPs) were prepared and coated with different NZs. Next, the resulting NZs-loaded Hb@MOF NPs (Hb@MOF/NZs NPs) were tested for their superoxide radical- and peroxide-scavenging abilities, with Amplex Red and WST-1 assays, respectively. Finally, the O_2 binding capacity of Hb@MOF/NZs NPs was measured and compared to that of free Hb with a needle-type O_2 microsensor.

RESULTS: The results for the enzymatic activity indicated that the Hb@MOF/NZs NPs displayed SOD- and CAT-like activity, with the former activity being superior.

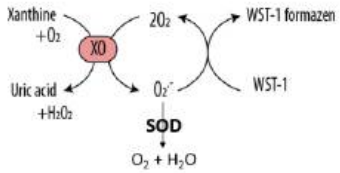

Figure 1: SOD-like activity of Hb@MOF/NZs NPs. O_2 is converted into $O_2^{\bullet-}$ by the xanthine/XO system. $O_2^{\bullet-}$ oxidizes the WST-1 reagent into formazan, which is detected by UV-vis. In presence of SOD-like activity, $O_2^{\bullet-}$ is consumed, decreasing the amount of oxidized formazan.

The O_2 release and binding studies showed that the Hb@MOF/NZs NPs assembled with lower concentrations of NZs present higher O_2 binding capacity.

DISCUSSION & CONCLUSIONS: The Hb@MOF/NZs NPs were successfully synthesized and coated with the different NZs. Their ability to deplete superoxide anions ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) was demonstrated prior and following incorporation into the Hb@MOF/NZs NPs. An O_2 electrode was employed to assess the O_2 binding and releasing properties of the encapsulated Hb, and the results showed no significant difference compared to the free Hb. As a further step, the surface modification of the NPs is proposed, to improve their stability and then continue further with biological evaluation in-vitro. In summary, these data demonstrate the potential of the as-prepared HBOCs as O_2 delivery systems with built-in antioxidant protection against reactive O_2 species.

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PLL coated CaP NPs as nanocarriers for DNA delivery

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INTRODUCTION: Nucleic acid vaccines have gained significant attention, especially after the development of COVID-19 mRNA vaccine. DNA vaccines are safe, inexpensive and can be rapidly designed and produced. However, challenges like the inefficient in vivo delivery and cell uptake limit their application [1]. The development of a more precise and potent vaccine platform is therefore needed.

Nanocarriers display unique features in protecting biomolecules from degradation, targeting the delivery, and controlling their release [2]. Calcium phosphate nanoparticles (CaP NPs) are one of the most promising DNA delivery platforms because of their biocompatibility and biodegradability [3]. In addition, CaP has a high affinity for DNA and has been widely used for DNA transfection studies.

To enhance the transfection efficiency and protect DNA from early degradation, polymers are often used in nano-vaccine formulations. Due to its cationic nature, polymerized lysine (PLL) can interact electrostatically with cells, facilitating efficient internalization for intracellular delivery[4]. Here we present a vaccine formulation, using CaP NPs for the loading of DNA which further is modified with polylysine as uptake enhancer.

METHODS: CaP nanoparticles were generated through a scalable single-step process employing flame spray pyrolysis (FSP). FSP is a commonly used industrial technique due to its high reproducibility and scalability. The CaP NPs were characterized using the Brunauer-Emmett-Teller (BET) method to evaluate the specific surface area (SSA) and X-ray diffraction (XRD) for crystallinity. The lysine polycondensation was conducted at 240°C; as at higher temperatures the amino acid degradation takes place. Transfection experiments were conducted in human embryonic kidney cells (HEK-293T) by delivering the linear vector of EGFP (enhanced green fluorescent protein), which was later analyzed via flow cytometry and confocal microscopy.

RESULTS: We synthesized amorphous CaP NPs confirmed with XRD, with a specific surface area of 230 m^2/g. We achieved more than 80% DNA loading efficiency on bare CaP NPs. DNA loaded CaP nanocarriers were formulated with and without polylysine for transfection studies. The PLL coated CaP NPs demonstrated enhanced DNA uptake by cells indicated by increased GFP production.

DISCUSSION & CONCLUSIONS: The utilization of calcium phosphate nanoparticles coated with lysine polymers showcased improved DNA uptake by cells, followed by the corresponding protein production. This presents a promising foundation for developing an effective DNA delivery platform.

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ACKNOWLEDGEMENTS: This work received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (ERC Grant Agreement No. 758705). Funding from the Karolinska Institutet, the Swedish Foundation for Strategic Research (SSF) (FFL18-0043, RMX18-0043) and the Swedish Research Council (No. 2021-05494, 2021-02059) is kindly acknowledged.

Porcine skin-derived hydrogels as bioink

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INTRODUCTION: The extracellular matrix (ECM) provides biochemical and structural support to the surrounding cells in living tissues. Comprising proteins and polysaccharides arranged in an intricate porous network, ECM incorporates cells, enzymes and growth factors [1]. Recreating its complexity in vitro, in terms of composition, structure, and mechanical properties, is a challenging task. The use of 3D bioprinting to fabricate 3D constructs with a hierarchical architecture similar to the native tissue could meet some of these challenges. Bioinks hence need to be formulated to give the biochemical cues for cell adhesion and proliferation [2], which may be missing in commonly used polymers. Decellularized ECM (dECM), obtained from the decellularization of ECM, is a promising candidate to that respect. dECM gels preserve the composition of ECM and thereby contain protein domains essential for cell stimulation [3]. However, 3D bioprinting using dECM gels is often unsuccessful due to their slow gelation kinetics and low mechanical properties. The concentration of dECM hydrogels could be a key parameter to formulate printable dECM bioinks. Here, we investigate how the formulation of skin-derived dECM hydrogels affects their gelation and viscoelastic properties, and therefore their printability and further use as bioinks.

METHODS: The dECM material was obtained from the decellularization of porcine skin tissue. Briefly, the tissue was first delipidated in acetone before several washing steps with surfactants, oxidant solution, buffers and ultrapure water. The quality of decellularization was assessed through biochemical assays. The dECM was then digested with pepsin at acidic pH for 24 h before adjusting the pH at 7.4 to obtain gels with several dECM concentrations. Gelation kinetics at physiological pH and shear viscosity were assessed with rheology measurements. The printability of dECM hydrogels was observed visually and with optical microscopy.

RESULTS: The decellularization removed the DNA while maintaining the levels of collagen and glycosaminoglycans in the dECM. dECM hydrogels with a storage modulus ranging from ca 100 Pa to 5 kPa were obtained after 10 min at 37 °C for concentrations of dECM up to 10 mg.mL^{-1} (Fig. 1a,b). The more concentrated bioinks can be extruded with a 30 G needle at speed up to 1000 mm.min^{-1}, and the prints presented a good shape fidelity (Fig. 1c).

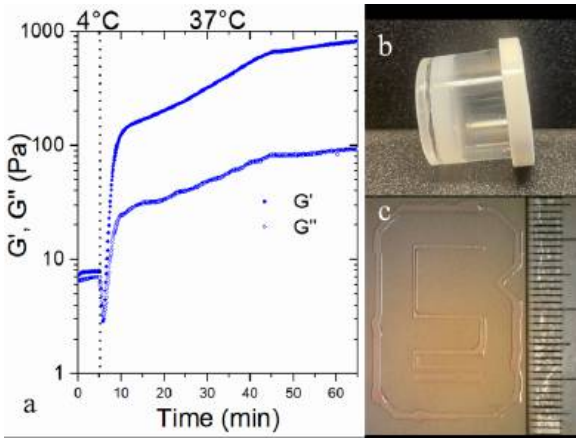

Fig. 1: a) Gelation kinetics of dECM hydrogel at 10 mg.mL^{-1}, b) picture of dECM hydrogel after gelation, c) printability test using a 10 mg.mL^{-1}dECM bioink (needle 30 G, 600 mm.min^{-1})

DISCUSSION & CONCLUSIONS: In this study, we prepared porcine skin-derived hydrogels. The gelation kinetics and moduli were comparable with other studies for gels obtained from various decellularized tissues. The gels at 10 mg.mL^{-1} may even compete with pure collagen hydrogels. These gels were also extrudable, which suggests the possibility to further process them by extrusion-based 3D printing. Thanks to the similarity of dECM with native tissues, dECM hydrogels have great chances to promote cell adhesion and proliferation. Further studies to fabricate 3D printed constructs are ongoing, together with biocompatibility studies. These constructs may be used for tissue engineering or in vitro tissue modelling.

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Optimization of an air-liquid interface system using electrospun membranes to mimic human airway epithelium for development of next-generation high-throughput airway model.

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INTRODUCTION: History of pandemics within the last century has been dominated by respiratory diseases, with COVID-19 being the most recent, making them remain a long-term public healthcare concern. As such, establishment of a new airway model is imperative to facilitate advancement in airway disease drug development. Air-liquid interface (ALI) is an established culturing technique which has been shown to have higher *in vivo* relevance compared to regular 2D cell culture, owing to its higher degree of biological and structural complexity [1] and electrospinning-based scaffolds [2] has been shown to have beneficial effects to cellular growth and maintenance due to its closer to *in vivo* topography. Here, we established the feasibility of combining both approach as a potential model amenable to high or medium-throughput screening (HTS) for lead compound identification purposes in primary human airway epithelial cells.

METHODS: We first characterized the optical transmittance of the PCL membranes in the visible light range using high-content plate reader and subsequently established proof-of-principle using a live-cell, far red fluorescent dye to monitor monolayer formation on the membrane. Different surface modification methods and ECM protein coating were utilized and the surface energy were tested using water contact angle (WCA) measurement. Next, we tested different methods to minimize the evaporation due to the "edge-effect" in 96- electrospun transwell plates.

RESULTS: On the visible light range, the lowest optical density was recorded at 630 nm wavelength. The used live-red dye were able to be used to visually track cell distribution on the membrane and treatment with 0.1% atelocollagen I were shown to be able to reduce the water contact angle (mean±SD = 75.125±1.536) Combination of breathable tape, liquid reservoir, and custom-made humidification chamber were able to reduce edge-effects by 71.50% (CI 95%=64.13 – 78.87).

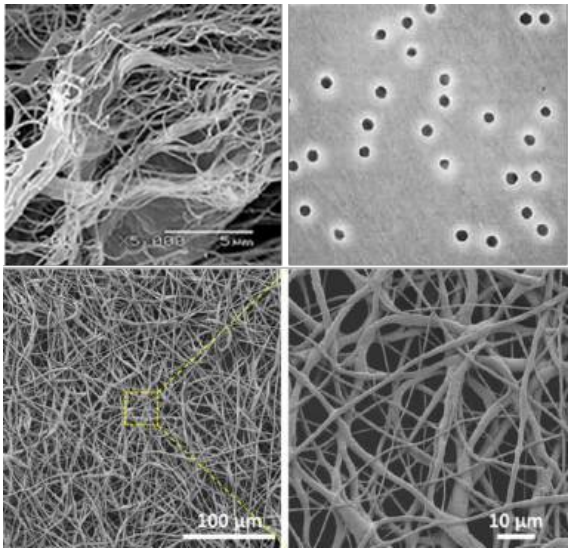

Fig. 1: Comparison between de celled airway basal membrane (Bridge et al 2015 top left) with commercial culture insert membrane (top right) and an electrospun PCL membrane (Tas et al 2021 bottom)

DISCUSSION & CONCLUSIONS: These early proofs of principles give hints on how to work with the electrospun PCL membrane for designing a high throughput drug screening model. Utilization of physiologically and clinically more relevant cells such as human airway cells may be implemented in the future to evaluate the proper biological response towards the material.

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Simultaneous delivery of cannabidiol and insulin-like growth factor 1 for tissue regeneration

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INTRODUCTION: Oral soft tissue regeneration after ablative surgery, trauma or oral cancer is still a challenging goal. Oral cancer is primarily treated with surgery, combined with chemotherapy and radiation, causing a significant loss of soft tissue, particularly gum [1]. The main problems for soft tissue regeneration are cell ingrowth, inflammatory and related pain issues. The wound-healing process includes cell migration, proliferation and differentiation, and the key signaling molecules that regulate these responses are growth factors. Insulin-like growth factor 1 (IGF-1) has an essential role in inducing cell proliferation and collagen synthesis in the fibro-proliferative process [2]. Furthermore, in recent years, medical cannabis has gained significant attention for its potential therapeutic benefits. Phytocannabinoids, such as *cannabidiol* (CBD), is known to speed healing of the wounds [3] and to relieve pain in chemotherapy [4]. The novel combination of IGF-1 and CBD could increase tissue regeneration and decrease chemotherapy-induced side effects. The delivery of both IGF-1 and CBD will be achieved through liposomal systems – by encapsulating them into liposomes. The aim of this work is to encapsulate both bioactive molecules into phospholipid-based liposomes and to evaluate their potential for simultaneously providing anti-inflammatory and antioxidant effect (by CBD) and inducing cell proliferation and collagen synthesis (by IGF-1).

METHODS: Liposomes were synthesized using cholesterol and various phospholipids (DSPE-PEG-Amine (MW3400), DSPC and DPPC) with thin-film hydration method. CBD was incorporated during synthesis process but IGF-1 – during hydration process of the liposomes. The chemical composition of liposomes was characterized by Fourier transmission infrared spectroscopy and the particle size and zeta potential was determined by dynamic light scattering analysis. The release of CBD and IGF-1 was determined using ultra performance liquid chromatography [5] and ELISA method, respectively. The *in vitro* cytotoxicity tests were conducted with gingiva-derived mesenchymal stem cells (GMSC) isolated from human patients according to the decision No. 6-1/12/47 (26.11.2020) of Riga Stradiņš University Research Ethics Committee. CCK8 assay was used for cell viability determination.

RESULTS: The particle size of liposomes varies between 3-9 µm and depends on the phospholipids used in the synthesis. The encapsulation of CBD and IGF-1 alter the particles size as the presence of CBD reduces the particle size. Based on the FTIR results, the synthesis process does not affect the chemical structure of the bioactive molecules. The cell viability for all samples (pure liposomes, with CBD, with IGF-1 and with CBD and IGF-1) is not lower than 70%.

DISCUSSION & CONCLUSIONS: Based on the cell viability results, all investigated samples are considered as non-cytotoxic, according to the ISO 10993-5:2009. The highest cell viability was observed for liposomes containing DSPE-PEG-Amine (MW 3400). Liposomes with IGF-1 show increased cell proliferation due to the mitogenic effect of IGF-1 [6].

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Rapid Manufacture of Engineered Muscle Platforms via In-Air Printing of Elastomer Composites

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[2] *Novo Nordisk Foundation*

INTRODUCTION: Conventional muscle microphysiological systems (MPSs) designed for drug screening and disease modeling rely on measurements of electrophysiological parameters and contractile forces, as muscle tissues exhibit both electrical and mechanical activity [1]. However, the current manufacturing process for muscle MPSs involves multiple steps[1] and limits the design flexibility. Direct Ink Writing (DIW) is a valuable 3D printing technique that has been proven to enable the simple production of a variety of complex geometries using appropriately engineered materials [2, 3]. This study proposes to explore the use of DIW as a rapid manufacturing modality for muscle MPSs. We have formulated an elastomeric composite conductive ink utilizing styrene-ethylene-butylene-styrene (SEBS) polymer and graphite microparticles in butyl acetate (ButAc) solvent. We have demonstrated that this material can be 3D printed in air, forming suspended filaments over several centimeters while retaining elasticity and conductive properties. As a proof of concept, we have used embedded additive manufacturing to bioprint C2C12 cells around two consecutive filaments in a sacrificial matrix. The resulting engineered muscle system holds promise for monitoring cell contractility.

METHODS: The DIW 3D printing method was used to print both the conductive ink and the muscle tissue. The graphite-based ink was characterized mechanically through tensile tests, electrically through multimeter measurements, and printability-wise through experimental prints and brightfield images. Tissue constructs were analyzed using brightfield images as well as actin-stained fluorescent images on a confocal microscope.

RESULTS: We developed an in-air 3D-printable material that shows both elasticity and conductivity properties to be used in muscle contractility tracking. In our previous work, we demonstrated the possibility of printing SEBS/ButAc-based materials in-air over centimeters in length (Figure 1a). In this paper, we explored a composite with graphite, which was characterized both mechanically and electrically. Embedded bioprinting of C2C12 cells was proven to be a valuable method for the creation of connected tissue constructs in precise placements around the support structures (Figure 1b).

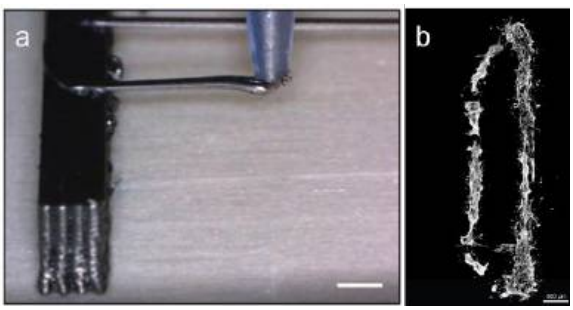

Fig. 1: (a) Free-standing in-air printing of the SEBS/graphite composite. Scale bar: 1 mm. Reproduced from[2]. (b) Confocal image of an actin-stained C2C12 tissue at 8 days in culture. Scale bar: 500 μm.

DISCUSSION & CONCLUSIONS: The SEBS/ButAc inks doped with graphite microparticles hold great promise in their ability to be free-suspended in addition to having both elastic as well as conductive properties. We believe that the combination of optimal solvent evaporation rate, particle gelation, and particle-polymer interactions allows the material to be printed in air without relying on support substrates. The embedded bioprinting allowed good localization of the tissue around the ribbons, paving the way for future electrical tracking of cell contraction.

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Microglial physiology changes in a stiffness-dependent manner in different 3D environments

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INTRODUCTION: Biomaterials have been used in research for decades as a tridimensional microenvironment for cell culture, being the acrylamide-bisacrylamide-based hydrogels the most widely used material. Many of these biomaterials do not have a biologically mimetic composition for the cell grow, in particular when modelling the brain parenchyma.

Microglia are resident macrophages of the brain that play an essential role in maintaining the homeostasis of the central nervous system (CNS) by sensing the environment.

Here we explore several biomaterials to achieve a more brain mimetic-like 3D-environment and studying whether matrix stiffness affect brain cell physiology in general and microglia in particular.

METHODS: We created 3D scaffolds with different methacrylated polymers, such as alginate, gelatine, and hyaluronan or chitosan, and using Matrigel as control biomaterial. We evaluated cell viability, phagocytosis, motility and morphology in SV40 microglia cells.

RESULTS: SV40 microglia are more viable in methacrylate alginate, gelatine and Matrigel. Alginate-based scaffold allowed microglia to adopt the more brain-like morphology, while softer Matrigel scaffolds allowed for increased motility.

DISCUSSION & CONCLUSIONS: Tridimensionality clearly affects microglial physiology and function, and it is in a 3D environment where brain cells can better mimic their in vivo counterparts. Whereas stiffness has a clear effect on movement and morphology, it is still not clear whether composition has a major effect on these parameters. However, composition and assembly protocol has a clear effect on cell viability. 3D scaffolds might prove a clear advantage over 2D cultures to better mimic the mechanical and chemical cues that cells find in the real brain, which might be useful for in vitro testing and screening

ACKNOWLEDGEMENTS: We thank the Microscopy Facilities at Achucarro Basque Center for Neuroscience and the UPV/EHU (Sgiker) for providing infrastructural support.

Fucoidan-Alginate Hydrogel Microbeads: Exploring Biological Responses

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INTRODUCTION: Fucoidans, which are branched and sulfated polysaccharides obtained from kelp, have garnered attention for their diverse bioactivities, with recent advances in the structural characterization of highly pure fucoidan [1]. Alginates, also derived from kelp, are well-known biomaterials, forming hydrogels under mild conditions for cells. Understanding host-biomaterial interactions is crucial for exploring potential applications of these hydrogels. In this work, we investigate fucoidan-alginate hydrogel microbeads implanted in mice to assess fibrotic responses and protein deposition by MS-proteomics. Additionally, we elucidate complement, cytokine, and coagulation responses in human whole blood.

METHODS: Fucoidan (F) and alginate (A) sourced from *Laminaria hyperborea* (*L. hyp.*) were utilized in this study. Hydrogel microspheres (≈ 400 to 500 µm) were generated at polymer concentrations ranging from 1.80% to 2.16% (w/v), with F to A ratios varying from 10/90 to 40/60. Implants in mice (C57BL/6JRj) and assays with human whole blood (HWB), as well as MS-proteomics, were conducted following procedures outlined in our previous research [2, 3].

RESULTS: Fibrotic responses (PFO) to microbeads containing fucoidan (F) revealed variable depositions, dependent on the ratio of F and A.

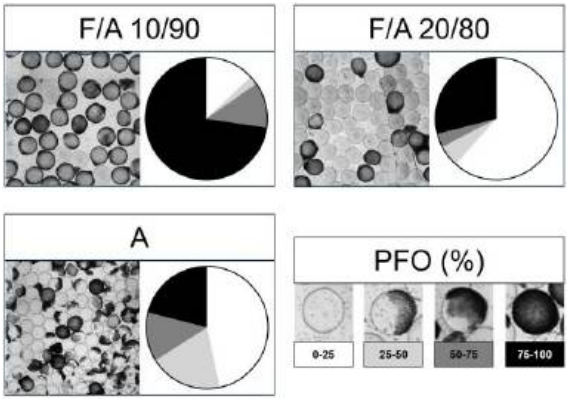

Fig. 1: PFO on F/A and A microbeads explanted from mice (n =3, 14 days).

A HWB assay unveiled that F/A microbeads interact with the coagulation cascade (measured by PTF1.2) and activate the complement system (measured by TCC), as illustrated in Fig. 2. Cytokine responses (not shown) remained low for F/A microbeads. Preliminary analyses of protein deposition on explanted microbeads indicate diverse binding, primarily of an anti-inflammatory nature.

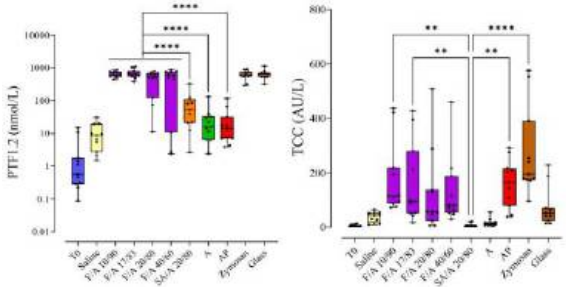

Fig. 2: F/A beads, controls, and relevant control microspheres (SA, sulfated alginate bead and AP, alginate-poly-L-lysine capsule).

DISCUSSION & CONCLUSIONS: Our studies reveal a nuanced pattern of bioactivity for fucoidan from *L. hyp.* in alginate hydrogels. In summary, it does not seem to exert anti-fibrotic effects, or this effect may be influenced by the concentration of fucoidan in the bead. While activating the complement system, overall cytokine secretion in response to F/A beads appears to be low. Moving forward, we aim to conduct more comprehensive analyses of protein depositions to gain mechanistic insights and uncover relevant applications for fucoidan in biomaterials.

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Save Your Tears for the Assay: Carbon Nanotubes Still Fooling Scientists

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INTRODUCTION: Carbon nanotubes (CNTs) are widely used material in different fields of industry and medicine due their specific properties. Because of their large-scale use, possible toxic effects on humans and the environment have been extensively studied. A common method for assessing the toxicity of CNTs are different cell viability assays based on tetrazolium salts, such as MTT assay. The international standard ISO 10993-5 also describes MTT assay and related tests for *in vitro* cytotoxicity testing. However, it has been observed that some of the assay dyes may interact with CNTs, leading to potential false results [1,2]. The issue of interactions between cell viability assay molecules and carbon nanomaterials remains to be investigated.

METHODS: We observed spectrophotometrically interactions between multi-walled carbon nanotubes (MWCNTs) and six different coloured tetrazolium salts: MTT, MTS, INT, XTT, WST-1, and WST-8. In metabolically active cells, tetrazolium salts reduce to formazan crystals by NAD(P)H-dependent oxidoreductase enzymes. Therefore, we utilized the formazan forms of dye molecules and cell-free systems to exclusively observe interactions between assay dyes and MWCNTs.

RESULTS: We found that all tested tetrazolium salt dyes interacted with MWCNTs, as evidenced by coloured dye molecules being absorbed into them. At a high concentration of MWCNTs, the absorbance intensity immediately starts decreasing at the beginning of each assay, and the intensity continues to decrease over time.

DISCUSSION & CONCLUSIONS: Our results demonstrate that cell viability assays are not reliable methods to study toxicity of CNTs. It is necessary to carefully investigate which *in vitro* methods are truly suitable for CNTs. For this purpose, it is important to study the interactions between carbon nanomaterials and cell viability assay molecules.

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Drug to polymer conjugates – a novel use of polyanhydrides in drug delivery systems

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INTRODUCTION: Polyanhydrides, e.g. poly(sebacic anhydride) (PSA), are considered drug carrier materials and have been used in clinical practice [1]. Recently, PSA was found to be promising in the delivery of azithromycin to the lungs in the form of dry powders for inhalation [2]. The success of the study was based on almost complete encapsulation efficiency. The phenomenon was explained by the creation of ester bonds between the polymer and the drug. Herein, we present a detailed mass spectrometry study on the establishment of drug-polymer conjugates with hydrophobic drugs with hydroxyl or phenol groups, i.e. azithromycin (AZM) and curcumin (CU), respectively.

METHODS: PSA was obtained by melt polycondensation. Microparticles (MPs) were manufactured by oil-in-water (O/W) emulsification, where O is a drug (either AZM or CU) and PSA dissolved in dichloromethane in various ratios, and W is a poly(vinyl alcohol) solution in MilliQ water. For CU-loaded MPs, the W-phase was additionally acidified with acetic acid, hydrochloric acid, or sulfuric acid. The morphology and size distribution of the MPs was assessed by optical and fluorescent microscopic observations and laser diffraction-based size measurements. The encapsulation efficiency (EE) and drug loading (DL) of AZM-loaded MPs were assessed by mass spectrometry (MS) of the supernatants, while for CU-loaded MPs, it was evaluated by fluorometric measurement after decomposing MPs in dimethyl sulfoxide. For analysis of the conjugates, the MPs were dissolved in chloroform, diluted 100 times, and injected directly into the mass spectrometer to detect the ions of conjoined drugs and monomers. The analysed ions were separated and fragmented to ensure their conjugate origin. To check the potential disturbance of the process on the bioactive properties, AZM-loaded MPs were tested with *Staphylococcus aureus* and the release of the pure drug in biological environment was tested by incubation with human microsomes.

RESULTS: The MPs obtained were spherical with most diameters in the range of 1-3 µm however, they showed a tendency to agglomerate resulting in median sizes between 10-30 µm in the form of dry powder. CU-loaded MPs were yellow due to the presence of the drug and exhibited a green glow when observed under the fluorescent microscope. Encapsulation evaluation showed that EE of AZM was nearly 100% while for CU-loaded it was up to 70% which was increased compared to around 10% for non-acidified conditions. MS analysis discovered conjoined drug molecules with up to several monomers at a time. Isolation and fragmentation confirmed their origin. Incubation with human microsomes showed gradual detachment of the conjugates in the biological environment, and AZM-loaded MPs effectively inhibited the growth of *S. aureaus.*

DISCUSSION & CONCLUSIONS: Hydrophobic drugs with hydroxyl or phenol groups can be encapsulated into poly(sebacic anhydride) with high efficiency due to the creation of drug-polymer conjugates; however, phenols require a low pH of water phase. The drugs do not lose their properties during the manufacturing process and can be released as pure substances in the human body. Furthermore, the issue of excessive agglomeration should be addressed in future studies.

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In vivo applications of Poly(glycerol sebacate urethane) Scaffolds

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INTRODUCTION: Soft tissue engineering (TE) is a multidisciplinary field with the aim to regenerate or replace a dysfunctional or damaged tissue. Usually, a three-dimensional porous scaffold is produced to provide a temporary structure and mechanical support while the tissue grows and subsequently replaces it. For this reason the scaffold should be highly tunable, with interconnected porous structure, be biocompatible, biodegradable and ideally to degrade linearly at the same rate as the tissue develops In this study, three PGSU scaffolds, with different mechanical and microstructure properties were fabricated and investigated for in vivo for their microstructure, biocompatibility and their ability to degrade at the same rate as tissue develops into the scaffold.

METHODS: PGSU scaffolds with hexamethylene diisocyanate (HDI) ratios of 0.8 and 1.0 and polymer concentrations (w/v%) equal to 10% and 15%. Briefly, the PGS pre-polymer was dissolved in 1,4-dioxane at the required w/v concentration and HDI was added at 0.8 or 1.0 ratio to glycerol. The solution was left to react for five hours at 55ºC. The solution was then frozen in an in-house customized mold (see 2) and freeze dried for 16 hours. In vivo biocompatibility of the scaffold was investigated by implanting scaffolds subcutaneously in CD1 albino mice for six weeks. Specifically, in total 16 mice had scaffolds implanted subcutaneously, 12 of which were implanted with four scaffolds of each sample group and the rest of them with Ethilon® Nylon suture 4-0 spheres which acted as the positive control. The scaffolds were also characterized for their microstructure using SEM. Finally, to assess any inflammatory response, whole body imaging (WBI) was used after

RESULTS: Figure 1A and B show the cross-sectional microstructure of the PGSU scaffolds before and after in vivo implantation. It was found that the previously open pore microstructure was filled with new tissue, demonstrating uniform tissue ingrowth. Subsequently, as the density increases the porosity decreases, in combination with the decrease in volume and no change in mass it can be said that the scaffolds degraded by surface erosion and the tissue ingrowth into the scaffolds is similar to their rate of biodegradation. Finally, no inflammatory response was observed during the in vivo imaging (see Figure 1C and D), which shows that the scaffolds are biocompatible and their degradation by-products do not cause any additional immune response.

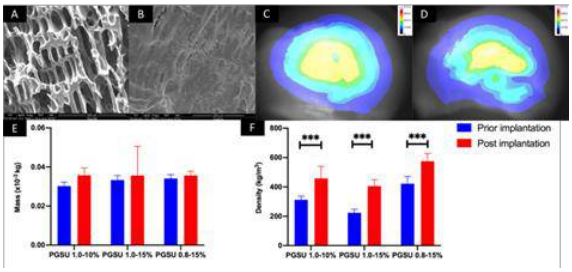

*Fig. 1: (A) Representative cross section microstructure of a PGSU scaffold prior implantation, (B) and 42 days post implantation; (C) whole body imaging of the mouse with the positive control, (D) and of the mouse with PGSU scaffold 42 days post implantation; (E) mass and (F) density of the PGSU scaffolds prior and post implantation. *** when p < 0.001.*

DISCUSSION & CONCLUSIONS: Large PGSU scaffolds were synthesized, fabricated and implanted subcutaneously into a mouse model for six weeks. The scaffolds demonstrated excellent integration with the surrounding tissue, and it promoted tissue ingrowth. At the same time, the scaffolds began degrading at a similar rate to the tissue development which is the ideal characteristic of a scaffold for the purpose to replace a damaged tissue. PGSU was shown in vivo to be biocompatible, biodegradable and promote tissue ingrowth at a rate that synchronizes with its degradation.

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Assessing Mechanical Degradation of Porcine Tissue

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INTRODUCTION: Autologous micrografting, a theory proposed by Meek in 1958, describes the concept of increasing the surface area (i.e. wound edges) of graft tissue to increase the rate of wound expansion [1]. Previous work has examined the expansion potential of micrografts fragmented from centimeters to millimeters [2]. Little is known, however, about how fragmentation on a micrometer scale can promote proliferation. This study aims to evaluate the proliferative potential of porcine endothelial micrografts when mechanically degraded to the micrometer scale.

METHODS: Porcine bladder harvesting: Tissue samples were collected from adult, female pigs used for a butchering course at ZBC Roskilde. After euthanization, bladder samples were collected through a midline incision and immediately washed in PBS (phosphate buffered saline, Sigma-Aldrich, St. Louis, US). Samples were transported on ice and suspended in 1×DMEM (Dulbecco's Modified Eagle Medium, Sigma-Aldrich) and antibiotics (penicillin 50 U/mL, streptomycin 50 µg/mL, and amphotericin B 2 µg/mL, Invitrogen, Thermo Fisher Scientific, Waltham, US). The bladder walls were pinned to sterile cutting boards to isolate the epithelium, which was cut into small fragments of approximately 2 mm^2 size and used in experiments or plated as control groups.

Mechanical degradation: Tissue dissociation was achieved using the FFX TissueGrinder (TG, Fast Forward Discoveries GmbH, Frankfurt, Germany). Pre-washed and cut tissue was loaded with 500 µL 1×DMEM into the inner circle of the TG's rotor. The stator and 40 µm cell strainer were placed on the rotor and enclosed in a 50 ml Falcon tube. The grinding process parameters were modified from the manufacturer's protocols (Table 1).

Table 1: Steps of the TG protocol used to process porcine tissue.

Process	Speed (rpm)	Duration (sec)
Cutting	+ 8	30
Grinding	- 8	30
Cutting	+ 15	40
Grinding	- 15	40
Cutting	+ 10	30
Grinding	- 10	30

After the protocol, the Falcon tube was inverted, the rotor was removed from the stator and sieve, and 100-200 µL of remnants were collected from the filter. The sample was resuspended and pipetted into 12-well plates for analysis and culture.

RESULTS: The imaged tissue, taken directly after degradation by the TG, show a heterogeneous fragment population (Fig. 1).

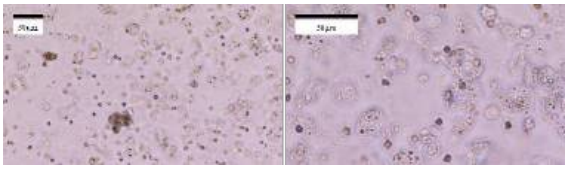

Fig. 1: Microscope images of processed tissue taken with 20X (left) and 40X (right) objectives on Day 0.

The tissue fragment sizes were estimated in ImageJ [3], assuming they were roughly spherical, to calculate the size distribution (Fig. 2).

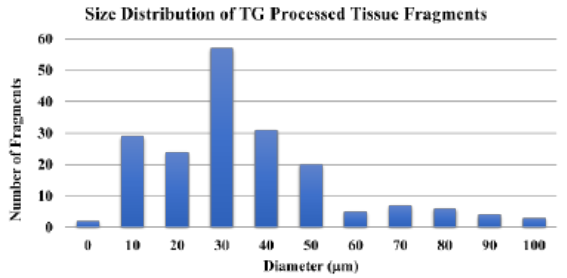

Fig. 2: Distribution of the diameter of processed tissue fragments up to 100 µm.

After nine days, endothelial cells and fibroblasts were observed in wells of TG samples (Figure 3) and little to none were observed in control groups.

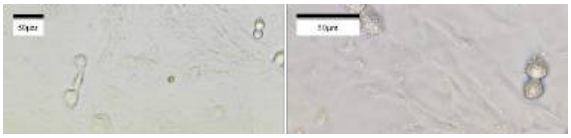

Fig. 3: Microscope images of processed tissue taken with 20X (left) and 40X (right) objectives on Day 15.

DISCUSSION & CONCLUSIONS: Porcine bladder epithelium mechanically processed by the TG showed rapid dissociation and proliferation in culture. Further investigation is needed to assess the reproducibility of this method with porcine bladder epithelium and additional tissues.

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RGD-alginate beads as a structure element for fibroblast culture

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INTRODUCTION: Hydrogel microparticles have been suggested as structure forming elements in granular hydrogels [1]. For alginate, a linear polysaccharide from seaweed, microbeads can easily be formed at physiological conditions by dripping alginate solution into a solution of divalent cations. To enable interaction with cells, alginate is commonly grafted with cell adhesion peptides such as RGD. To enable high grafting densities, we first oxidized the alginate before subsequent grafting by reductive amination [2]. We used the RGD-grafted alginate for the formation of microbeads and investigated the ability of granular hydrogels to support the adhesion and growth of normal human dermal fibroblasts (NHDFs).

METHODS: High MW alginate with a high content of guluronic acid (68 % G, 237 kDa) (UPLVG, NovaMatrix, Norway) was oxidized to 8% before grafting with GRGDSP peptide (Caslo, Denmark) with reductive amination [2]. A 5% degree of substitution was characterized by ^{1}H-NMR. 2 % (w/V) alginate was used for the formation of beads of about 500 µm by using an electrostatic droplet generator and a gelling solution of 114 mM NaCl and 10 mM $BaCl_2$. NHDFs were seeded on top of a bilayer of microbeads in 96 well plates at a concentration of 25 % oxidized alginate grafted with GRGDSP (POA-RGD) and cultured at 5% O_2 and 37ºC. At day 3, the cells were fixed and stained for nuclei (DAPI), cytoskeleton (Phalloidin) and immunolabeled with ki-67 monoclonal antibody, before visualized by confocal scanning microscopy (CLSM).

RESULTS: NHDFs adhered to the microbeads with RGD and spread on the bead surfaces (Fig. 1, day 3). The cells interconnected with cells on the neighboring beads. A large proportion of the cells showed proliferative activity through the expression of the ki-67 marker. In contrast, microbeads without RGD did not result in cell adhesion to the beads (not shown).

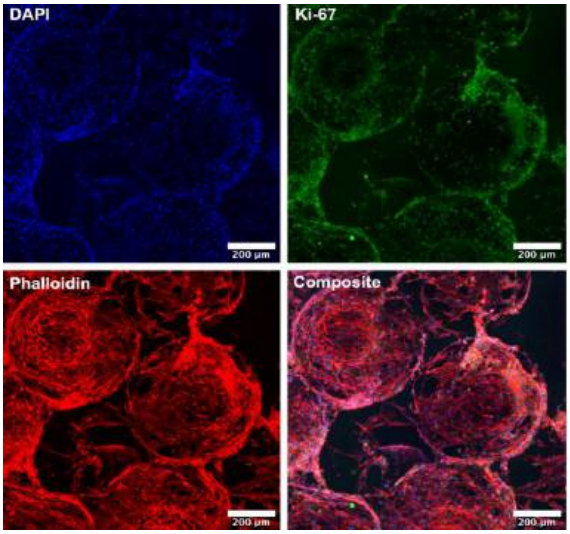

Fig. 1: NHDFs were fixed and labeled with DAPI (blue), ki-67 antibodies (green) and Phalloidin (red) after 3 days of culturing with 25 % POA-RGD microbeads.

DISCUSSION & CONCLUSIONS: Alginate is an excellent material for the formation of regular microparticles due to the formation of spherical hydrogels by dripping into a solution of divalent ions. Here, RGD alginate was used in the formation of beads that allowed the adhesion of NHDFs and cellular interconnections between cells on different beads. Hence, the RGD-beads are promising materials as structure elements for the culture of cells.

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ACKNOWLEDGEMENTS: We are grateful for funding from NTNU Health.

Comparative Study of Coating Textile Knitted Scaffolds with Human Bone Powder vs. Hydroxyapatite Powder for Bone Regeneration

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INTRODUCTION: Hydroxyapatite (HA), which closely mimics the structure of natural bone mineral, has been extensively researched for its potential in bone regeneration [1,2]. The mineral has shown promise in enhancing the osteoconductive properties of implant surfaces, making it a focal point in the field of orthopaedic and dental implants [1]. The present study aims to contribute to this body of research by conducting a comparative investigation into the coating of textile scaffolds with human-derived bone powder and commercially available HA powder. The primary objective is to explore potential differences in the source of the mineral and its implication for bone regeneration.

METHODS: Knitted spacer fabric made of Poly(lactic acid) (PLA) monofilament were coated with powder derived from human femoral heads or commercially available hydroxyapatite (< 200 nm, Sigma-Aldrich Co) in an ethanol (EtOH) solution using an ultrasonic bath. Prior to the coating, the knitted PLA scaffold underwent surface activation using 1M NH_3. The distribution and characteristics of the coating were analysed using scanning electron microscope (SEM) and Fourier transform infrared spectroscopy (FTIR). Subsequently, the cellular attachment was assessed through *in vitro* screening of bone marrow-derived human mesenchymal stem cells (hMSCs) at various time points using fluorescence and confocal microscopy. The differentiation of the hMSCs into osteoblast was evaluated by measuring the alkaline phosphate activity (ALP).

RESULTS: The findings of this study demonstrate the uniform distribution of the coating on the knitted PLA scaffold, as confirmed by SEM and FTIR analysis. Furthermore, the knitted PLA scaffold when coated with human-derived bone powder or HA, supported the attachment and the proliferation of hMSCs (Fig. 1). Additionally, the comparable osteoconductive properties of HA and human-derived bone powder were substantiated through ALP activity assessment.

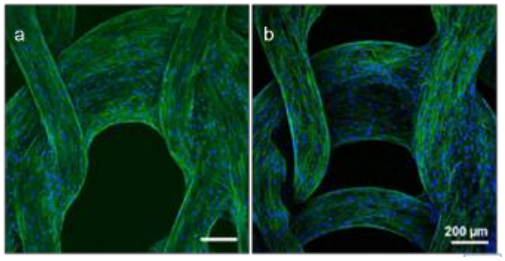

Fig. 1: Morphology of hMSCs cultured in osteogenic induction medium after 21 days as visualized by fluorescent staining of actin filaments (green) and nuclei (blue, a) Human derived bone powder and b) HA coated knitted PLA scaffold. Bar = 200 µm

DISCUSSION & CONCLUSIONS: The results from this study revealed that both human-derived bone powder and commercially available HA effectively enhanced the osteoconductive properties of the knitted PLA scaffold. Notably, no significant difference was observed between the two apatite powders in terms of their osteoconductive effects. However, a notable distinction was identified in the particle morphology of the two types of powder. Specifically, the commercially available HA exhibited a spherical shape, whereas the human-derived bone powder displayed a more irregular shape. Given the substantial impact of particle morphology and topography on cell attachment and proliferation [1], it is advisable to further optimize the shape and size of the particles for a more comparable study in the future.

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Antimicrobial activity of Ag/SiO_2 flame-made nanoparticles

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INTRODUCTION: The global rise in antibiotic-resistant bacteria, primarily fueled by the overuse and misuse of antibiotics, presents a pressing public health concern, necessitating innovative approaches to combat infections. Nanoparticles, offer a promising approach against antibiotic-resistant infections. Silver nanoparticles (Ag NPs), due to their small size, are extensively studied for their ability to increase the bacterial outer membrane permeability, interact with intracellular components, and release Ag^+ ions, leading to destabilization and cell death [1]. Additionally, the incorporation of silica (SiO_2) into composite Ag/SiO_2 nanoparticles enhances their stability, prevents their agglomeration, and augments their antibacterial properties [2].

METHODS: In this study, Ag/SiO_2 nanoparticles were produced by flame spray pyrolysis (FSP), a scalable and reproducible aerosol-based method. Varied Ag content (0, 20, 40, and 60 %wt) was achieved while maintaining a constant total metal concentration. Thorough analysis involved specific surface area (SSA) measurement via N_2 adsorption, crystallite size determination using X-ray diffraction patterns, and structural analysis through Transmission electron microscopy. The release of Ag^+ ions at different incubation timepoints was quantified. Moreover, the *in vitro* assessment of antibacterial effectiveness against *Methicillin-resistant Staphylococcus aureus* (MRSA) involved colony-forming units (CFUs) enumeration method (Fig. 1), covering nanoparticle concentrations ranging from 12.5 to 100 µg/ml. To evaluate practical application in physiologically relevant context, NPs *ex vivo* testing was performed on porcine skin wounds infected with MRSA. As final formulation, the nanoparticles were incorporated into 4% w/w methylcellulose solutions. The antimicrobial activity and zone of inhibition against MRSA were assessed using the disk diffusion assay.

RESULTS: Ag/SiO_2 nanoparticles demonstrated a notable reduction in CFUs/ml compared to SiO_2, positioning them as promising nanoantibiotic agents. The study reports a dose-dependent antibacterial efficiency of Ag/SiO_2 nanoparticles, with enhanced antibacterial efficacy observed in nanoparticles with higher Ag content. Remarkably, Ag/SiO_2 nanoparticles displayed antibiotic effects comparable to, and in some cases, surpassing those of *vancomycin*—an established MRSA antibiotic.

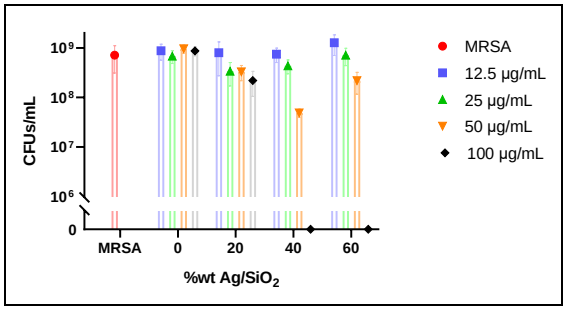

Fig. 1:Mean values and standard deviations of the number of MRSA CFU/mL (log 10), obtained by the CFU enumeration method of xAg/SiO2 NPs treatment, in four different concentrations.

DISCUSSION & CONCLUSIONS: Overall, this study underscores the heightened potential of nanoparticle-mediated therapeutic strategies utilizing inorganic nanoparticles in the fight against antibiotic-resistance infections. These findings hold promise for addressing the critical issue of antibiotic resistance, both in controlled laboratory settings and within physiologically relevant conditions.

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Enhancing Hemoglobin-Based Oxygen Carriers: Insights from Tannic Acid Interaction and Metal Ion Influence

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INTRODUCTION: In addressing challenges associated with the standard transfusion of donor blood, including limited availability, disease transmission risk, and delays due to blood typing requirements, the development of hemoglobin-based oxygen carriers (HBOCs) has emerged. HBOCs, leveraging hemoglobin (Hb), aim to replicate the oxygen-carrying capacity of blood without the constraints of blood typing. Despite attention on encapsulating Hb in micro- and nanosized carriers, achieving high Hb loading remains a challenge [1]. A recent one-step self-assembly method in water, incorporating polyethylene glycol (PEG), phenolic ligands, metal ions, and bioactive macromolecules, presents a promising approach to synthesize functional protein nanoparticles, addressing drawbacks in conventional blood transfusions [2]. Notably, phenolic ligands, beyond their role in assembly, can interact with Hb, potentially altering its structure and function. Controlling these structural changes can offering potential improvements in product efficacy.

METHODS: Utilizing fluorescence spectroscopy, we investigated the interaction between Tannic acid (TA), a key phenolic component in current Hb nanoparticles (Hb-NPs), and Hb. The nature of this interaction was probed using the Stern-Volmer and van't Hoff equations, while computational analysis provided deeper insights. Hb-NPs were synthesized through a one-pot assembly method, incorporating various concentrations of PEG, manganese (Mn^{2+}), and TA into a Hb suspension. The impact of different Mn^{2+} concentrations on Hb functionality was examined using an oxygen meter, revealing potential alterations in Hb functionality due to increased interaction opportunities between TA and Mn^{2+}.

RESULTS: Upon the addition of TA, fluorescence emission spectra exhibited quenching. Analysis using the Stern–Volmer equation unveiled a static quenching process dominated by the formation of a compound between Hb and TA. The apparent binding constant (K_A) increased with temperature, indicating robust and temperature-stable binding, with a probable single binding site near the β-37 Trp residue. Thermodynamic analysis disclosed spontaneous and endothermic binding, primarily propelled by hydrophobic interactions. Docking simulation depicted TA's preference for interacting with hydrophobic residues of Hb, particularly the β-37 Trp residue, emphasizing its capability to trap Hb clusters through hydrophobic interactions. The preservation of Hb's function was observed by increasing Mn^{2+} concentrations.

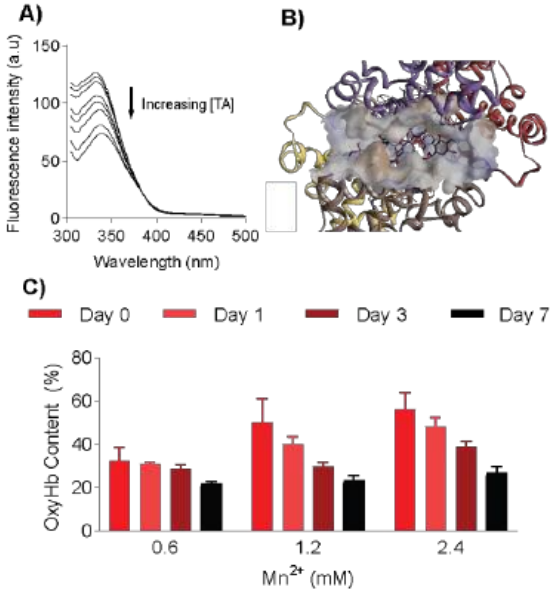

Fig. 1: A) TA quenching effect on intrinsic Hb fluorescence. B) Docking simulation of Hb and TA. C) OxyHb content of Hb-NPs fabricated using three different concentrations of Mn^{2+}.

DISCUSSION & CONCLUSIONS: While Hb-NPs consistently preserved Hb's functionality through multiple oxygenation and deoxygenation cycles, the O_2 loading capacity was influenced by Mn^{2+} concentration. Higher Mn^{2+} concentrations led to an augmented oxyHb content, proposing a potential effect from reduced TA availability to interact with Hb at elevated Mn^{2+} levels.

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ACKNOWLEDGEMENTS: We express our gratitude to 3B's group members at DTU for their valuable collaboration during this research.

Coaxial nanofibrous wound dressing with antibacterial and photothermal flame-made nanoparticles

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INTRODUCTION: Nanofibers have found significant applications in diverse fields such as tissue engineering, drug delivery, wound dressings, energy cells, and textiles. Among the various techniques that exist to produce nanofibers, electrospinning offers a significant advantage of tuneable fiber characteristics such as diameters and morphologies, while being cost-effective. Coaxial electrospinning is a modification of this technique that can be used to fabricate core-sheath nanofibers [1]. They can be used for encapsulating cargo with a controlled release rate. Here we have developed a core-sheath nanofibrous anti-bacterial wound dressing. The sheath consists of silver-silica nanoparticles (Ag/SiO_2 NPs), which possess anti-bacterial characteristics [3] that targets wound infections- a phenomenon that significantly impedes the healing process. On the other hand, the core contains photothermal gold-silica (Au/SiO_2 NPs) that provide localized heating in response to infrared radiation- a process known to aid in wound healing [2].

METHODS: Ag/SiO_2 and Au/SiO_2 NPs are produced using flame spray pyrolysis (FSP) where silver acetate and gold acetate respectively are used as precursor materials alongside a combination of acetonitrile and 2-ethylhexanoic acid [4]. These particles are further incorporated into core-sheath fibers using the electrospinning setup Fluidnatek LE-50. The heat response was obtained using a thermal camera (Ti480 Pro, Fluke) under laser irradiation at 808 nm.

RESULTS: The presence of both the antimicrobial Ag/SiO_2 NPs as well as the photothermal Au/SiO_2 NPs renders the core-sheath fibers multifunctional. We demonstrate the anti-bacterial effect against Methicillin-Resistant Staphylococcus Aureus (MRSA)- a pathogen known to cause wound infections. Further, we validate the photothermal effect of the membrane by near-IR laser irradiation.

DISCUSSION & CONCLUSIONS: Wound infections and removal of wound dressings remain critical challenges to overcome in this domain. We present a step towards this by producing a core-sheath nanofibrous membrane using the cost-effective industrial manufacturing processes of flame spray pyrolysis and electrospinning. The membrane initially targets the wound infections using the anti-bacterial particles encapsulated in its sheath while the photothermal particles in the core structure enables localized heating which is known to assist in wound healing.

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Development of Multifunctional Self-Healing Sensors for Enhanced Wearable Health Monitoring

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INTRODUCTION: Wearable health monitoring devices due to the variety of qualities that they offer have become the prime focus in modern health care. These bioelectronic devices are flexible, skin conforming, portable, durable, highly sensitive, and can provide real-time monitoring.

They have proved to be highly useful in numerous cases such as patients going through the rehabilitation process, in the evaluation of the post-surgery performance of athletes, translation of sign language for individuals with inability to hear, early diagnosis and prognosis of certain illnesses such as Parkinson disease and also in sports performance and human-machine interfaces for robotics [1]. The strain sensors can also be widely applied in sports performance and human-machine interfaces for robotics. Flexible wearable sensors are the key point for monitoring motion and physiological signals and daily activity. The current studies on flexible and stretchable strain sensors have hardly succeeded to cover the important characteristics of the next generation of wearable electronics for rehabilitations [2]. These features involve low cost, high sensitivity, high elasticity, self-healing capability, and multi-functionality. Personalized cheap and accurate wearable electronics for human motion monitoring can help patients and doctors for rehabilitation.

METHODS: Here a sensitive, self-healable, and elastic strain sensor is developed by embedding nanomaterials along with a conductive polymer, PEDOT PSS. The sensors are sensitive to strain, pressure, and temperature and can achieve high stretchability up to 600% and great durability due to the self-healing properties.

RESULTS: The electrical properties, sensitivity, and mechanical properties of the materials were tested at different strains. The conductivity of the materials goes up to 10^{-3} S/cm while showing sensitivity to different temperatures between 15 ^{o}C to 60 ^{o}C based on the mechanical test. It can heal itself more than 95% in less than 3min. Sensitivity to pressure was measured too and it shows significant changes in conductivity when a wide range of pressures from 1kPa to 1MPa was applied to the sensor.

DISCUSSION & CONCLUSIONS: In this study, we developed a low cost and accurate and self-healable sensor for healthcare monitoring. We expanded the functionality of the material to also monitoring temperature of the body. This sensing material was also highly durable because of self-healing properties.

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Lactic acid grafted microcrystalline cellulose loaded membranes for guided bone regeneration

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INTRODUCTION: Cellulose based biomaterials exhibit important properties such as porosity, elasticity that makes them exciting for guided tissue regeneration (GTR) application [1]. Biomaterials for guided tissue regeneration need to have a balance between material stiffness, elasticity, and flexibility to maintain their barrier property. On the other hand, they should maintain their structural properties such as porosity, biocompatibility and have antibacterial properties [2]. Most of the reported GTR specific biomaterials/membranes compromise structural properties such as porosity for strength and typically lack antibacterial properties. The current work focuses on the development of lactic acid (Lac) biofunctionalized microcrystalline cellulose (MCC) reinforced membrane for GTR. .

METHODS: The MCC was biofunctionalized through lactic acid (Lac) grafting (MCC: Lac = 1:20, 1:23, 1:25, 1:30 w/w) and they were characterized based on physical and chemical properties using Fourier-transform infrared spectroscopy (FT-IR), zeta potential and scanning electron microscopy (SEM). The membrane was prepared by solvent casting by mixing different ratios of MCC-Lac with chitosan-aqueous acetic acid solution where 1.5% w/v chitosan was present. Other polymers like polyethylene glycol (PEG, 5% w/v) was also mixed and stirred for 10-15 min.In this mixture, genipin (0.07% w/v) is given as a biosafe crosslinking agent and stirred for 5-8 min. The polymer solution was then solvent casted in petri plates and incubated at 24 h at 37 ºC. Finally, MCC-Lac reinforced chitosan membrane was obtained.

RESULTS: Lactic acid biofunctionalized MCC was successfully prepared through grafting. The degree of functionalization and zeta potential of the grafted samples are as follows: MCC-Lac: 1:30 > MCC-Lac: 1:25 > MCC-Lac: 1:23 > MCC-Lac: 1:20. The SEM and FTIR study indicated notable grafting of lactic acid on the surface of MCC. The MCC-Lac= 1:30 samples was utilized to prepare chitosan-based biofunctionalized membrane. The prepared membrane was freeze-died and characterized on the basis of physiochemical properties. The FTIR analysis indicated the successful grafting of Lac on MCC, and presence of chitosan. The membrane also exhibits notable internal porosity. Additionally, SEM study indicated significant porous structures. (Fig. 1).

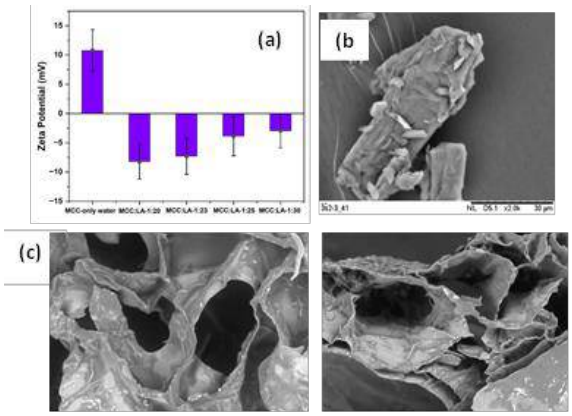

Fig. 1: (a) Zeta potential and (b) SEM of biofunctionalized MCC: Lac=1:30 and (c) MCC-Lac= 1:30 reinforced chitosan based membrane.

DISCUSSION & CONCLUSIONS: MCC is being considered as a significant reinforcement filler material [3]. Lac has antibacterial property. The major objective of biofunctionalization of MCC with Lac is to prepare highly efficient reinforcement filler with the antimicrobial property for the developed membrane. MCC-Lac: 1:30 exhibited high degree of biofunctionalization that is indicated through zeta potential, FTIR analysis. The high degree of biofunctionalization resulted from the higher degree of Lac grafting. Hence, this sample was used to prepare the membrane. The SEM images of the membrane indicated significant macroporous structure. These are important for infiltration and growth of cells which is necessary for the guided tissue regeneration.

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Textile scaffolds of P(3HB)/P(3HB-co-4HB) blend filaments

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INTRODUCTION: Tissue engineering is a multidisciplinary field which aims to replace damaged or diseased tissue by artificial implants. Textile technology with its precise control of yarn placement and control of pore size can be one option to produce tissue engineering scaffolds [1]. Besides structural integrity through controlled yarn placement, textile technologies result in controlled anisotropy, mimicking the natural tissue [2]. The success of a textile-based scaffold does not only depend on its design but also the filaments and its properties that built up the final scaffold [3].

Poly(3-hydroxybutyrate) (P(3HB)) and poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P(3HB-co-4HB)) are biopolymers with excellent biocompatibility and degradation behaviour making them interesting candidates for biomedical applications [4].

This poster investigates the melt spinnability of P(3HB)/P(3HB-co-4HB) blend filaments and their degradation in isotonic media comparable to the human body. Additionally, the filament's potential for textile-based scaffold formation is investigated.

METHODS: The filaments were produced by melt spinning of a P(3HB)/P(3HB-co-4HB) blend (57 mol%/ 43 mol% with a 4HB content of 30 mol% in the copolymer). Tensile testing and differential scanning calorimetry (DSC) was conducted on undrawn filaments before and after seven weeks immersion in phosphate buffered saline solution (PBS) at 37°C. The PBS had a pH value of 7.4 and was changed weekly. To investigate the processability of the filaments a tube was knitted on a small circular knitting machine.

RESULTS: The filaments possessed a degree of crystallinity of around 53% as well as a tensile strength of 21.5 ± 2.3 MPa at an elongation at break of 341 ± 168% and a Young's modulus of 862 ± 168 MPa before the immersion in PBS. Seven weeks after immersion in PBS, no gravimetric weight loss could be detected. However, especially the filament's elongation at break deteriorated dramatically from around 341% to 6.7% after being exposed to PBS. The tensile strength decreased by around 5.5 MPa whereas the Young's modulus increased by 178 MPa. Additionally, DSC showed a slight increase of crystallinity to 60%. To investigate the processability of the filaments into a textile, a small tubular knit was produced (Fig. 1).

Fig. 1: Tubular knitted structure from P(3HB)/P(3HB-co-4HB) blend filament.

DISCUSSION & CONCLUSIONS: To our knowledge, there are no other reports of P(3HB)/P(3HB-co-4HB) blend filaments, thus the mechanical properties before degradation are compared to pure P(3HB-co-4HB) filaments with a 4HB content of 4 mol% [5]. Our filaments are in average 5 times stiffer while exhibiting a 1.5 times higher elongation at break as compared to the P(3HB-co-4HB) filaments. Only the tensile strength of our filaments is half of the pure P(3HB-co-4HB) filaments which can be explained due to the lack of filament drawing and thus molecular alignment in our case. A filament drawing would of course influence the other mechanical parameters and the degree of crystallinity. The deterioration of mechanical properties during aging is because of the faster degradation of the amorphous segments as the crystalline parts of the polymer, which mainly contribute to tensile strength, have a higher resistance to hydrolysis [6]. This is reflected by the increased tensile strength and degree of crystallinity of our filaments and in alignment with Vodicka et al.'s results during the degradation of P(3HB-co-4HB) in artificial body fluids [6]. The textile processability of the filament is shown by the knitted tube, which is promising for the pending textile-based scaffold.

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ACKNOWLEDGEMENTS: The authors would like to acknowledge Maria Persson for fruitful discussions and Stefan Gustafsson for knitting the sample.

Automated 3D Printing of Micro-structured Substrates for Rapid Generation of Anisotropic Skeletal and Cardiac Muscle Tissues

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INTRODUCTION: Anisotropic organization of cells and extracellular matrix is a common hallmark for several types of mammalian tissues, not least striated muscle[1,2]. In the absence of unidirectional cellular alignment skeletal myofibers developed in vitro from myoblasts thus tend to be orders of magnitude shorter than their in vivo counterparts. Similarly, cardiac muscles engineered in vitro that are not anisotropic fail to replicate the end-to-end electrical coupling through gap junctions between cells, which is a crucial aspect of in vivo myocardial electrophysiology[3]. Anisotropy is therefore an essential feature to replicate when producing physiologically relevant models of cardiac as well as skeletal muscle. Several methods have been introduced for producing tissue culture substrates with microscale cues to direct the self-assembly of tissues into anisotropic architectures[4,5]. However, methods such as microcontact printing and micro-molding are time-consuming and laborious due to their processing times and manual steps. Here, we show a rapid and automated 3D printing procedure for fabricating micro-structured tissue culture substrates. The method relies on the fabrication and use of macroscopic printing nozzles containing micro-scale tip features that are transferred to the extruded surface during deposition. The procedure therefore relies on the shear-thinning and thixotropic properties of the ink materials.

METHODS: The conventional plastic nozzles were patterned through a hot embossing process on a silicon wafer featuring defined micro-sized ridges. The resultant patterned nozzles were employed in the 3D printing of extrudes, facilitating the transfer of structures onto the surface of the extrudes during the printing process. Subsequently, both human and murine cells were seeded on the patterned substrates, and the alignment of the cells along the established structures was evaluated through microscopy imaging.

RESULTS: The examination of the nozzle end and extruded surface by using optical profilometer measurements proved the successful replication of patterns from the wafer onto the substrates. Consequently, the cells cultivated on these structured surfaces exhibited the formation of anisotropically aligned monolayers.

DISCUSSION & CONCLUSIONS: We demonstrate that in-situ micro-patterned substrates provide sufficient topographical cues for generating unidirectionally aligned cultures of striated muscle tissue. We also demonstrate how micro-structured nozzles for the procedure can be conveniently produced in a low-tech setup by hot embossing of conventional plastic nozzles. Our method provides a route for automated fabrication of complex culture substrates and devices while decreasing the overall fabrication time.

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Optimizing Alginate Blends for Stable Microbead Formation with Controllable Size

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INTRODUCTION: Microencapsulation of biologics using biomaterials aims to secure and protect bioactive compounds for controlled release and is being extensively investigated across the pharmaceutical, cosmetics, and food industries. Hydrogel beads, especially those containing encapsulated agents, have gained considerable attention. Alginate, a naturally occurring polymer, has garnered widespread use in these technologies owing to its availability, cost-effectiveness, biodegradability, and biocompatibility. Different methods for microbead formation using alginate have been reported such as electrically driven droplet generators, emulsification, and microfluidics. However, their ultimate utility for certain applications, such as in encapsulating cells and molecules, is affected by the physical and mechanical characteristics of the resulting alginate hydrogel. Alginates used for the fabrication of hydrogels differ based on their composition (i.e. guluronic (G) and mannuronic(M)) and chain length. These parameters impact the stability of the resulting hydrogel based on the crosslinking approach used. However, the use of single types of alginates can be a limiting factor as the availability of alginates with both the chain length and G/M ratio needed for specific applications can be limited or cost-prohibitive. Our research aimed to optimize a blend of two alginate types (low and high molecular weight (LMW and HMW) and low/high G/M to fabricate alginate beads that exhibit mechanical and structural stability when fabricated using a water/oil emulsion fabrication system.

METHODS: The emulsion of water in oil protocol [1] was employed alongside Ca-EDTA internal gelation [2], utilizing a design of experiments approach to determine the optimal ratio of different sodium alginates and crosslinking approach. Acetic acid in oil was used to induce internal gelation via calcium release from Ca-EDTA or Ca-CO3.

RESULTS: A combination of 4% LMW, high G/M and 1% HMW, low G/M with 50 mM Ca-EDTA resulted in significantly more stable microbeads than individual use, even at higher concentrations. Stirring speeds of 1000 rpm produced >80% circular microbeads with a diameter of <500µm. 2% v/v acetic acid in oil for 10 minutes was sufficient for crosslinking and internal gelation.

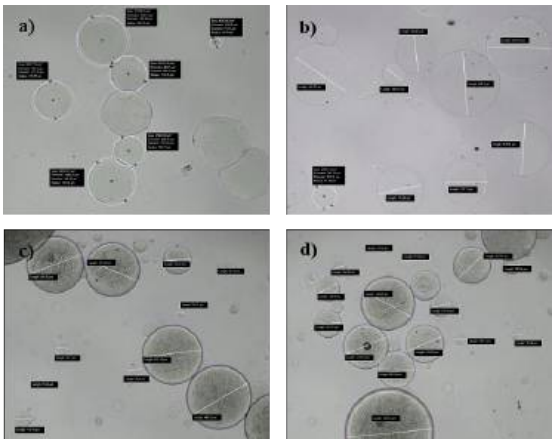

Fig. 1: Sodium alginate microbeads: a) LFR 4%; b) CR 1%; and c and d) LFR 4%+CR 1%.

DISCUSSION & CONCLUSIONS: A combination of alginate characterized by a high G/M ratio and HMW facilitates the creation of stable and flexible microbeads. Employing Ca-EDTA as a calcium source instead of the commonly used CaCO3 enhances solubility without inducing CO2 release in the gelation process. A brief pH change during internal gelation could be an effective strategy for preventing cell death in cell encapsulation but requires further optimization. Next, we will implement this protocol within a microfluidic system for cell encapsulation to illustrate its applicability for cell transplantation.

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PEGDA-Based PolyMIPE and Hyaluronic Acid Stabilized Gold Nanoparticles Composites for Bone Tissue Engineering

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INTRODUCTION: Polymerized medium internal phase emulsions (PolyMIPEs) are macroporous materials have attracted interest in tissue engineering as highly porous scaffolds to support cell proliferation. The polymerized high internal phase emulsions (PolyHIPEs) developed through water-in-oil (W/O) emulsions are usually hydrophobic in nature [1] and therefore in this work we aimed to use medium internal phase emulsions (MIPEs) to increase the hydrophilicity by using a hydrophilic polymer poly (ethylene glycol) diacrylate (PEGDA) through an oil-in-water (O/W) emulsion. To improve the bioactivity and cell attachment to the scaffolds we loaded the scaffold into the hyaluronic acid (HA) coated gold nanoparticles (HA-AuNPs).

Gold nanoparticles have demonstrated promising applications in tissue engineering due to their ease of synthesis, size controllability, surface plasmon resonance (SPR), and biocompatibility [2]. Recently, it was discovered that AuNPs can regulate cell differentiation and promote the regeneration of bone and cartilage tissue [3].

In this study, we synthesized a PEGDA-based polyMIPE composite incorporating HA-AuNPs. The resulting composite scaffold will be tested for its effectiveness in bone tissue engineering.

METHODS: The polyMIPE was fabricated via thermal polymerization of PEGDA in the internal phase at 70℃ for 24 hours followed by washing with ethanol and water and drying. HA-stabilized AuNPs were synthesized by in-situ reduction method, briefly, the gold chloride solution in DI water was refluxed for 2 hours then the temperature was brought down to 40℃ followed by the addition of HA- gallic acid (GA) solution with pH 8.5 and stirred at 40℃ for 12 hours. Then the porous scaffold was loaded into the HA-AuNPs as shown in Fig. 1.

RESULTS: Hydrophilic porous polyMIPE materials were obtained with improved antioxidant activity after loading with HA-AuNPs, which supports the cell attachment and proliferation. The HA-AuNPs were obtained from the HA stabilized via polymer mediated reduction method and were characterized by dynamic light scattering (DLS). The average particle size was 150 nm and zeta potential were -32. The composites of polyMIPE-HA-Au-NPs will be tested for bone tissue engineering.

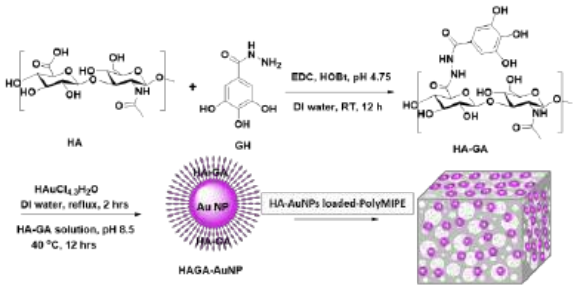

Fig. 1: Synthesis of HA stabilized gold nanoparticles and their composites with polyMIPE.

DISCUSSION & CONCLUSIONS:

The composites obtained from HA-functionalized AuNPs and polyMIPE displayed good antioxidant properties due to the presence of HA-AuNPs. In addition, polyMIPE exhibited good porosity, elasticity and swelling, which can be attributed to the emulsion's volume phase composition and the presence of hydrophilic PEGDA, respectively.

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Modification of Fluoride Ion Release Behaviour Dispersed in Dental Poly Methyl Methacrylate Material by Incorporating Polyethylene Oxide

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INTRODUCTION: Poly Methyl Methacrylate (PMMA) is a commonly used dental material, which is often used to make artificial teeth and splints [1]. Sodium fluoride (NaF) is a commonly used anti-caries drug [2]. Therefore, it is a good idea to add NaF to PMMA, which can prevent dental caries by releasing fluoride ions. However, NaF is water-soluble, while PMMA is hydrophobic. Hence, NaF could not disperse well in PMMA, and it is not conducive to the release of fluoride ions. Therefore, we designed a strategy to add Polyethylene Oxide (PEO) [3], an amphiphilic and biocompatible material, to the mixture of PMMA and NaF to improve the release behaviour of fluoride ions.

METHODS: 10 g of PMMA and 3 g of PEO were mixed in acetone, and then 1 g, 2 g and 3 g of PEO were added to the PMMA/PEO mixture, respectively. The mixture was dried to form thin films. The surface and cross-section of the films were observed by scanning electron microscopy, and their element distribution and functional groups were analysed by energy dispersive spectroscopy and Fourier-transform infrared spectroscopy, respectively. The surface roughness and contact angle of the films were also measured. The fluoride ion release rate of the films was tested by immersion experiments and their *in vitro* biocompatibility was tested by cytotoxicity experiments.

RESULTS: In the images of the film surface morphology, compared with the film without PEO, the NaF clusters on the surface of the film with PEO added were smaller and more dispersed. In the cross-section images, NaF clusters were not only embedded on the surface of the films, but also evenly dispersed inside the films. Similarly, the clusters inside the film without PEO were also larger than those inside the film with PEO added. And there was no phase separation or delamination phenomenon between PMMA and PEO.Fourier transform infrared spectroscopy showed that the spectra of films without PEO added and films with different amounts of PEO added had the same bands, and the intensities of these bands were close.

The average surface roughness test results showed that the average roughness of the films decreased from nearly 0.8 μm to about 0.2 μm after adding PEO. Static contact angle test results show that adding PEO can reduce the film surface contact angle, and the more PEO added, the smaller the contact angle.

The immersion test results showed that adding PEO could significantly increase the release of fluoride ions. After adding 3 g of PEO, the fluoride ion release amount increased nearly 20 times.

Cytotoxicity test results showed that although the cytotoxicity of films containing PEO was greater than that of films without PEO, it was within an acceptable range [4].

DISCUSSION & CONCLUSIONS: It can be proved that PMMA and PEO were uniformly mixed together in this study, while NaF was dispersed in the form of clusters through the scanning electron microscopy images and Fourier transform infrared spectroscopy. Moreover, the addition of PEO could enhance the dispersion of NaF clusters, hence the clusters became smaller and more uniform, which decreased the surface roughness further. Moreover, since PEO is an amphiphilic material, it also decreased the contact angle by adding PEO. The PEO mixed in the films might provide a channel for the internal NaF clusters to release fluoride ions, thus significantly increasing the release of fluoride ions. The released fluoride ions might cause some cytotoxicity, but fortunately the cytotoxicity was within an acceptable range [4].

Above all, this experiment provides a strategy to combine PEO with PMMA, a commonly used dental material, to improve the release behaviour of fluoride ions. It is expected to endow dental appliances with anti-cariogenicity.

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Effects of Temperature and pH in the Self-Assembly of Polyphenol Nanoparticles

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INTRODUCTION: Natural polyphenol molecules exhibit several pharmacological properties relevant for preventing and treating diseases: antioxidant, anti-inflammatory, anticoagulant, and anticancer [1]. However, these natural phenolic compounds possess low stability and poor water solubility, essential for their clinical applications [2].

This study aims to optimize polymer-based nanoparticles' *water-in-water* synthesis process and particle growth control. This will enable reproducible synthesis in pharmaceutical product development and ensure the efficacy of the drug-delivery systems.

METHODS: Poly-quercetin nanoparticles (pQu-FA NPs) were synthesized via a one-step polymerization process of quercetin and folic acid in PB solutions at varying pH values. The NP samples were stirred or sonicated at different time lengths. Then, the generated NPs were suspended in ddH_2O for freeze-drying and consequently dissolved in various conditions to test for particle stability.

UV-VIS spectrometry characterized the nanoparticles to identify the characteristic profiles of quercetin and folic acid. Dynamic light scattering (DLS) was performed to assess the size distribution profiles of the pQu-FA NPs. Fourier-transform infrared spectroscopy (FTIR) was also performed to determine surface chemical composition. Scanning electrode microscope (SEM) imaging was used to analyze NP stability. Furthermore, the cytotoxicity and antioxidant activity of pQu-Fa NPs were assessed *in vitro*.

DISCUSSION & CONCLUSIONS: The stability of nanoparticles is highly influenced by external factors such as pH, temperature, stirring speed, and stirring duration.

Preliminary experiments have shown that adding a zwitterionic molecule such as folic acid improves particle encapsulation efficiency and achieves a more homogenous NP solution and consistent size of NPs (**Figure 1**). Additionally, by combining FA with a PB solution of higher pH, the aggregation of NPs decreases, and the permeance of polymeric components is improved (as seen in SEM).

Finally, accomplishing a higher Z-potential heightens the stability of NPs when in suspension [3].

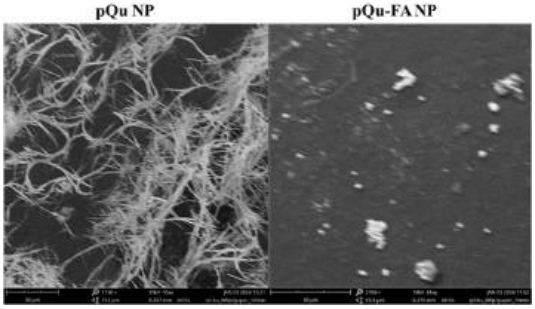

Figure 1: SEM images of polymeric nanoparticles with and without adding folic acid during synthesis.

Our results suggest that the particle size and stability can be controlled by adjusting pH, time, and stirring conditions. Moreover, upon encapsulation, particles show minimal toxicity *in vitro*, suggesting their future applications and research as pharmaceuticals.

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Hierarchically Porous Metal−Organic Frameworks-Based Hemoglobin Carriers with Surface Click-PEGylation: Boosting Colloidal Stability and Biocompatibility

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INTRODUCTION: Timely transfusion of red blood cells (RBCs) is an essential and life-saving procedure for avoiding body hypoxia and subsequent organ failure when patients suffer from acute severe blood loss. However, the limited supply and portability of donor RBCs and the time-consuming blood matching and typing, coupled with their short half-life and special storage requirements, make the opportune RBCs transfusion very difficult, especially during man-made or natural disasters. Incorporating hemoglobin (Hb), the central component of blood for oxygen transport, into nanoparticles to fabricate Hb-based oxygen carriers (HBOCs) can bring promising RBCs substitutes. HBOCs have potential for delivering oxygen and overcoming the abovementioned limitations when donor blood is not available, and incidentally addressing the tissue toxicity of free Hb [1]. Our group previously reported HBOCs by encapsulating Hb within mesoporous metal-organic frameworks (MOFs), PCN-333, and demonstrated their ability for Hb protection and oxygen carrying [2]. Nevertheless, achieving long circulation and biocompatibility are upcoming challenges when developing HBOCs. Meanwhile, expanding the variety of MOFs for macromolecule Hb pore encapsulation is also important since microporous MOFs predominate the reported structural repertoire.

METHODS: As shown as Fig 1A, herein we present a novel HBOCs based on hierarchically porous MOFs, in which the ligand was partially replaced with a sacrificial one with relative weak metal binding affinity, forming structural defective MOFs. After the removal of the sacrificial ligand, the generated mesopore provides appropriate cavities for Hb pore encapsulation. The obtained HBOCs were further functionalized with PEG coating.

RESULTS: The proposed hierarchically porous MOFs successfully encapsulated Hb, overcoming the natural pore size limitation of the pristine micropore MOFs structure (Fig 1B). The resultant HBOCs uniformly appeared as round-shaped particles sized around 190 nm (Fig 1C). Importantly, the surface PEGylation rendered the HBOCs with a significantly improved colloidal stability and stable hydrodynamic diameters compared to their parent counterparts, which is essential for upcoming *in vitro* and *in vivo* studies.

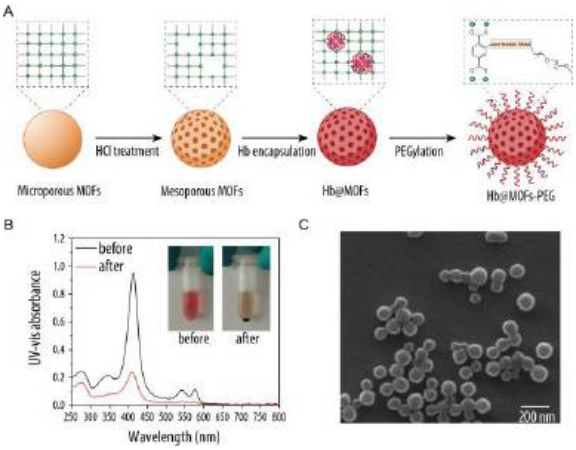

Fig. 1: A) Schematic illustration of the synthesis of PEGylated Hb-encapsulated MOFs; B) UV-vis absorbance of Hb solution revealed the encapsulation; C) SEM of Hb@MOFs-PEG.

DISCUSSION & CONCLUSIONS: These findings highlight the structural engineering of PEGylated hierarchically porous MOFs, providing new insights into the design of MOFs for Hb delivery.

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Cell membrane coated biomimetic nanoparticles for tumor targeting

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INTRODUCTION:

Cell membrane (CM)–coated biomimetic nanoparticles (NPs) have attracted a lot of attention in nanomedicines due to their unique biological features such as immune evasion, prolonged blood circulation, and homologous tumor targeting [1]. However, several key questions about the biomimetic NPs, such as CM coating integrity, are still not fully understood, preventing the further advancement of the novel coating technique for cancer therapy. The present study aims to thoroughly investigate the effect of CM coating integrity on tumor targeting [2].

METHODS:

The CMs of CT26 cells were first extracted using the Dounce homogenizer [1]. The CM vesicles and porous silica NPs were mixed with the same mass (protein weight for CM vesicles) and co-extruded through a 200 nm membrane to prepare the CM coated biomimetic NPs. A fluorescence quenching assay was proposed to quantitatively probe the integrity of the CM coating, which is a vital metric to assess the coating quality of the biomimetic NPs. At the end, a novel approach using external phospholipid was designed to improve CM coating efficacy (Fig. 1A). The NPs were characterized with physicochemical methods and evaluated with *in vitro* assays & *in vivo* animal experiments.

RESULTS:

Our results discovered that most of the biomimetic NPs (>90%) were only partially coated with CM [1], which contradicts the common assumption that the NPs would be completely coated. Furthermore, the mechanisms with molecular simulations revealed that the partially coated NPs were internalized into cancer cells through an aggregation-based cooperation mechanism. To address the problem of partial CM coating, the use of external phospholipid increased CM fluidity, promoting the final fusion of CM on the NPs. Consequently, the tumor targeting of the biomimetic NPs was significantly improved by enhancing CM coating efficacy (Fig. 1B) [2].

DISCUSSION & CONCLUSIONS:

NPs coated with CMs using non-optimized techniques have exhibited promising biomedical properties. To enhance the coating techniques for achieving better coverage of NPs, it is crucial to optimize the coating process and study the influencing parameters. This research offers innovative perspectives on cell membrane coating technology, paving the way for the strategic development of biomimetic NPs tailored for targeted cancer therapy.

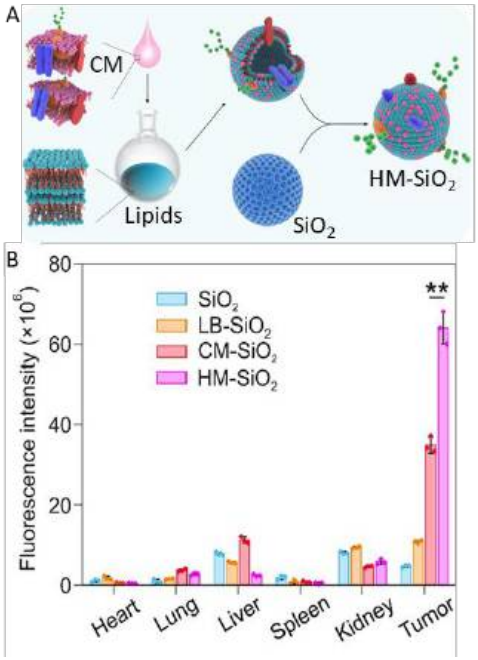

*Fig. 1. A) Schematic showing the process of developing hybrid membrane (HM) coated HM-SiO_2 NPs. B) In vivo tumor targeting by the analysis of the fluorescent signals from the tumor and major organs collected at 96 h after intravenous injection. **$p < 0.01$.*

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