AFPM

Advanced Functional Polymers for Medicine 2022

1-3 June 2022
CEMEF
Nice, France

Abstract Book

Advanced Functional Polymers for Medicine 2022

Welcome to the Advanced Functional Polymers for Medicine 2022 conference at the Center for Materials Forming of Mines Paris in Sophia Antipolis. Established in 2011 by professors Andreas Lendlein and Dirk Grijpma, the purpose of the AFPM conference series is to strengthen the interactions within the community of chemists, material engineers, physicists, biologists and clinicians in the development of Advanced Functional Polymers for Medicine. The current status, challenges and requirements for future developments of polymers for medicine are presented by leading experts. The conference provides an outstanding opportunity to help young scientists in their career development and offers them an interdisciplinary discussion forum within an exclusive circle. The AFPM 2022 conference will offer delegates innovative and stimulating topics with a well-balanced program of invited speakers and poster presentations.

Sophia Antipolis is an excellent place for our gathering. Created in 1969, Europe's largest science and technology park now hosts 2,500 companies and research institutions employing more than 38,000 people from over 80 nationalities. Sophia Antipolis is located close to the Mediterranean coast with its sunny beaches and vibrant cities of Nice and Antibes.

We warmly acknowledge the generous support of ours sponsors, who are featured on the next page. We also thank all invited speakers, poster presenters and other delegates for taking the time to travel to Sophia Antipolis and attend AFPM 2022 in person.

Should you have any questions, please ask the local organizing committee, who are portrayed below.

Bienvenue sur la Côte d'Azur!

Chairs of AFPM 2022
Dr. Sytze Buwalda, Mines Paris, France
Dr. Tatiana Budtova, Mines Paris, France
Prof. Andreas Lendlein, University of Potsdam, Germany
Prof. Dirk Grijpma, University of Twente, The Netherlands
Prof. Nicola Tirelli, Italian Institute of Technology

Local organizing committee AFPM 2022


Dr. Sytze Buwalda


Dr. Tatiana Budtova

+ numerous others (PhD students, administrative staff etc.)

Sponsors of AFPM 2022

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Wednesday June 1st

11:00	**Registration open**		Foyer
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14:00-14:30	Prof. Laurent Corté Mines Paris & ESPCI – PSL University, France	*Hydrogel-tissue adhesion: slippery when wet*	
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16:30-17:00	Prof. Christine Jérôme University of Liège, Belgium	*Non-isocyanate polyurethane networks: new opportunities for biomaterials*	
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12:00-12:30	Prof. Elisabeth Engel Institute for Bioengineering of Catalonia, Spain	*Role of biomaterials in endogenous tissue regeneration*
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Lecture abstracts

ordered chronologically according to the program

Mesenchymal Stem Cells Sense the Toughness of Interfaces

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Introduction

Stem cells sense and respond to a broad range of physical stimuli arising from their extra-cellular environment. In particular, mechanical properties of biomaterials have a significant impact on the phenotype of stem cells (1). In turn, cells exert forces on their environment that can lead to striking changes in shape, size and contraction of tissues, and may result in mechanical disruption and functional failure. However, the impact of biomaterials toughness on stem cell phenotype has not been explored to date. Here, we show how macromolecular architecture regulates interfacial toughness and how this controls the expansion of MSCs.

Experimental Methods

To modulate interfacial toughness, we assemble thin (10-20 nm) protein/polymer nanosheets displaying strong interfacial mechanical properties. This behaviour enables cell adhesion, expansion and the maintenance of stemness (2-4). We use interfacial rheology to quantify interfacial toughness and draw correlations with MSC expansion and phenotype.

Results and Discussion

In this report, we demonstrate that the molecular weight of the polymers used for assembly at liquid-liquid interfaces controls the toughness of the resulting nanosheets, independently of their interfacial shear mechanics. We propose that interfacial toughening is enabled by the bilayer structure of nanosheets (Figure 1), with extended soft segments enabling effective stress dissipation and preventing crack propagation.

In turn, we find resulting nanosheets enable cell adhesion and the long term expansion of MSCs at the surface of microdroplets (emulsions). Cells cultured in such way retain comparable phenotypes (CD73/CD90/CD105 triple marker expression, THY/NES/VCAM1 expression of and ability to undergo osteo/adipo/chondrogenic differentiation) to plastic grown cells.

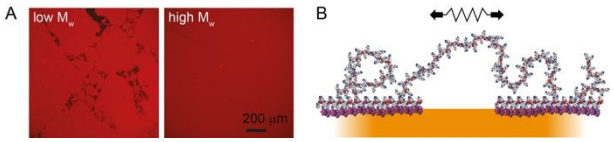

Figure 1. A) The toughness of nanosheets depends on their molecular weight. B) Stress dissipation by nanosheets during fracture.

Conclusions

Overall, our data demonstrate the importance of nanoscale and interfacial toughness on the adhesion and phenotype of adherent cells. We propose a simple mechanism via which interfacial mechanics and toughness can be rationally modulated. The system presented demonstrates the ability to culture stem cells over prolonged periods of time on microdroplets, paving the way to a new generation of bioreactors based on emulsions, and stem cell manufacturing platforms.

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Acknowledgments

Funding from the Leverhulme Trust (RPG-2017-229, Grant 69241), the ERC (ProLiCell, 772462) and the China Scholarship Council (201708060335) is gratefully acknowledged.

Hydrogel-tissue adhesion: slippery when wet

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The fixation of hydrogels to soft biological tissues is of outmost interest for numbers of biomedical applications but it is a highly challenging task due to the fragile and wet nature of both hydrogels and tissues. Here, we explore how physical mechanisms occurring at hydrogel-tissue interfaces can be exploited to design bioadhesive hydrogels that are relevant for clinical applications. For that, *ex vivo* and *in vivo* experiments were devised to measure the adhesion between model polyethylene glycol hydrogel films and the surface of porcine livers. We find that a transition from a lubricated contact to an adhesive contact is governed by the transport of liquid across the tissue-hydrogel interface. This transition is well captured by a simple model describing the competition between the wetting of the interface by the water coming from the tissues and its draining by the swelling hydrogel. By reducing *in vivo* the vascularization and exudation of the liver, we show that this effect explains the strong decrease in adhesion observed between *ex vivo* and *in vivo* conditions. These results suggest a new route to improve adhesion using superabsorbent hydrogel meshes. Inspired by the pioneering works by Leibler and coworkers, we then investigate how tissue-hydrogel adhesion can be created using particles that bridge the interface by adsorbing on both gels and tissues. As an example, for a 5 min contact on liver tissues, a 3 to 4 fold increase in adhesion energy was obtained by simply coating dry PEG membranes with aggregates of silica or iron-oxide nanoparticles. *Ex vivo* and *in vivo* experiments show how adhesion depends on the contact parameters, coating properties as well as on the hydration of tissues and on the presence of blood. These results and methods shed a new light on the design of predictive bioadhesion tests and on the strategies to control the fixation and biointegration of hydrogel based-devices.

Functionalized polysaccharides as antimicrobial agents

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Modified polysaccharides have a huge potential in several applications, and they exhibit substantial beneficial properties, where the antimicrobial activity is of great interest in various fields. Taking advantages of surface modification with permanent positive charge of quaternary ammonium compounds, this study aims to develop metal free and environmentally friendly materials, with excellent long-lasting antimicrobial activity. The material, based on modified polysaccharides[1], may have the potential to be used as hand/surface sanitizers, in textile (PPEs) and water treatment industries, or in various biomedical applications.

In this research, two quaternary compounds, glycidyl trimethylammonium chloride (GTMAC) and [2-(acryloyloxy)ethyl]-trimethylammonium chloride (AETMAC), were introduced to both chitosan and carboxymethyl chitosan structures, generating both single and double quaternized structures[2]. This research investigates how the structure, type of quaternary ammonium compound, functional groups of quaternized chitosan derivatives, and their density of positive charge can affect their antiviral or antibacterial activities. It is suggested that the viral behaviour of chitosan is also dependent on the hydrophobic interactions between the cell wall and the hydrophobic functional groups introduced to the chitosan. It is proposed that the higher hydrophobicity and longer alkyl chains of AETMAC influence the antiviral activity of quaternized chitosan, compared to GTMAC-derivatives.

The anti-viral behaviour depended on the type of virus, time of exposure and environmental conditions. Furthermore, these quaternized polysaccharides can be utilized to generate self-healing injectable hydrogels, with inherent antimicrobial properties, suitable for *e.g.* wound healing applications[3]. This research proposes quaternized polysaccharide-based materials as metal free and environmentally friendly alternatives to commercially available antimicrobial agents.

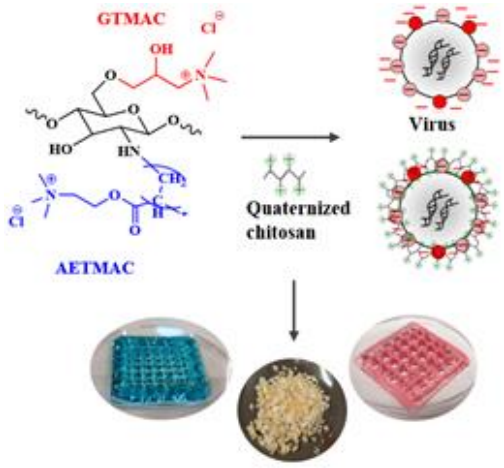

Figure 1. Chemical structure of quaternized chitosan with both GTMAC and AETMAC and their proposed viral interaction. The obtained product can, together with other polysaccharides, be formed into powder, films or make 3D printed structures.

References

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3. M. Madani, I.E. Laurén, J.V. Seppälä. 2022 (ongoing)

Acknowledgments

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Non-isocyanate polyurethane networks: new opportunities for biomaterials

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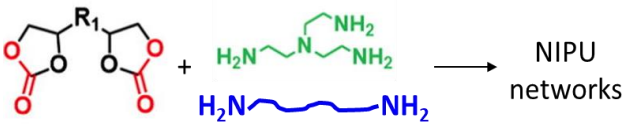
Introduction

Polyurethanes are versatile materials finding applications in various sectors including medicine. Currently, they are obtained by step growth polymerization of bis-isocyanates with polyols. Due to the toxicity of isocyanates, there has been an increasing interest to develop more environmentally-friendly and safer alternatives. The synthesis of non-isocyanate polyurethanes (NIPU) by polyaddition of diamines with CO_2-sourced bis(cyclic carbonate)s has emerged as one of the most promising strategy.

We investigated the development of novel conceptual routes for the synthesis of such isocyanate-free PUs by valorizing different CO_2-sourced building blocks, targeting mild conditions in order to disfavor the occurrence of side reactions. We particularly focused on the synthesis of polypropylene glycol (PPG) based NIPU elastomers by reacting a bis-cyclocarbonate PPG with various bis-amines and a triamine (scheme 1, R_1=PPG). Depending on the nature of the used amine and formulation composition, elastomers valuable for biomedical applications are foreseen.



Scheme 1. Synthesis strategy for NIPU networks

Experimental Methods

Telechelic bis-cyclocarbonate-PPGs (CC-PPG) were synthesized by coupling CO_2 to poly(propylene glycol) diglycidyl ether (640 g/mol and 380 g/mol) (Aldrich) and then reacted with various bis-amine.[1] A triamine (TAEA) was used as crosslinker. Formulations were mixed for 10 min then cured for 24 h at 70°C.

The networks were characterized by swelling tests in $CHCl_3$. Water-uptake was also measured. Tensile tests were performed at room temperature.

Results and Discussion

By varying the molar mass of the PPG precursor and the nature of the diamine, elastomers exhibiting high elongation at break (above 200%) and high tensile strength (1.4MPa) have been obtained by one step thermal curing at 70°C. Besides, by reacting the CC-PPG at both ends with allyl amine, a photoactive hydroxyurethane PPG is obtained which in presence of a trithiol and a photoinitiator is converted efficiently into performant elastomers. Given the fast UV curing conditions and the appropriate viscous behavior of these PPG-PHU based formulations, they were found suitable photoinks for 3D printing with a bioplotter.

Conclusions

By using an isocyanate-free process, PPG-NIPU elastomers have been obtained. These materials are promising candidates for applications as medical devices as supported by the first results of biocompatibility studies.

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Acknowledgments

The authors thanks the National Foundation for Scientific Research (FRS-FNRS and FRIA grant) and the DGO6 (Walloon Region) in the frame of the NIPUGEL project (Win2wal) for supporting this work.

Novel thermogelling and inverse thermogelling polymers for drug delivery and 3D (bio)printing

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Introduction

We have previously discovered library of diblock and triblock copolymers, which, when dissolved in aqueous media, forms a thermogelling or inverse thermogelling and highly shear-thinning hydrogels, respectively [1]. Such materials are of interest as injectable drug depots or for biofabrication, i.e. additive manufacturing of cell-ladden constructs.

Experimental Methods

Polymers were prepared by cationic ring-opening polymerization and characterized thoroughly using size exclusion chromatography and NMR spectroscopy. 3D printing was conducted using a BioX from CellInk AB.

Results and Discussion

The hydrogels exhibit excellent cytocompatibility and could be used for 3D printing with live mammalian cells (fibroblast) with high cell viability. However, while this hydrogel could be 3D printed, shape-fidelity and long term stability was not satisfactory. Addition of laponite XLG could be shown to significantly improve this [2]. Further, blending with alginate was investigated. After printing, the alginate could be crosslinked by simple addition of Ca^{2+} containing solutions, which at the same time, washes out the thermogelling polymer. The entire process, mixing, printing, crosslinking and washing was found to be highly cytocompatible [3]. Moreover, the hydrogel could also be mixed with PEG-diacrylate, which could be cured using two-photon polymerization, allowing freefrom laser writing and creation of microscopically small topologically connected 3D "chain-mail" like hydrogel. Such structure are of interest for advanced cell culture and tissue engineering [5].

Conclusions

The combination of different hydrogel forming polymers as presented here drastically expands the application profile of hydrogels for biomedical application, in particular in 3D printing.

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Acknowledgments

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Development of a macromolecular platform to yield functional degradable networks with actuation, self-healing and/or bioadhesion properties

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Introduction

Degradable 3D-networks, like elastomers and hydrogels, are largely spread among biomedical applications owing to their potentials for use in medical devices, like surgical patchs, or as drug delivery systems, like subcutaneous therapeutic depot[1]. However, 3D-networks are generally only designed with mechanical performances in mind. Other important properties like self-healing, degradation, bioadhesion and so forth are not easily accessible. In this contribution we report on single macromolecular platform based on degradable star-shaped copolymers that tackles this limitation by offering multi-functionalities. In more details, families of 8-arms PEG)$_{S8}$-PLA star block copolymers are designed to yield 1) bilayered constructs with actuation properties, 2) self-healable elastomers or 3) bioadhesion properties.

Experimental Methods

8-arms PEG)$_{S8}$-PLA star block copolymers (SBC) with defined hydrophobic/ hydrophilic balance have been prepared according to previous works of our group.[2,3] Bilayered self-rolling patches have been prepared from methacrylated elastomers and hydrogel SBC precursors. Self-healable elastomers and resorbable bioadhesives have been prepared from SBC dually-functionalized by methacrylate (MC) or cathecol (CT) groups, or combination of both. Instron 3344 testing machine was used to assess the mechanical, self-healing properties (self-healing efficiency SHE, time of repair) and bioadhesion properties. SEM was used to characterize the morphologies and optical visualization was used to characterize self-healing and actuation properties. Biocompatibility and degradation of the various SBC-based biomaterials was evaluated *in vitro* under standard conditions.

Results and Discussion

PEG)$_{S8}$-PLA star block copolymers with tunable hydrophilicity were obtained under scale-up friendly conditions allowing the production of up to 100 g. Quantitative chain ends functionalization were confirmed analyses for all targeted functional groups. For the self-rolling patches, elastomer and hydrogel precursors were photo-crosslinked under UV to yield a 500µm thick bilayered construct. No delamination was observed as a result of the similar nature of the precursors. The gradient of swelling between the layers led to self-rolling in less than 30s upon hydration. Unrolling was obtained upon dehydration. Drug loading and elution was evaluated with an anti-inflammatory drug. Self-healable elastomers with 100% SHE was obtained with MC_{75}/CT_{25} blends and the dually-functionalized $MC/CT_{75/25}$ elastomers. Bioadhesion properties between the ones of fibrin and cyanoacrylate surgical glues were confirmed for CT- and MC-functional hydrogels on mucus models. Colon adhesion was also tested.

Conclusions

Degradable and 3D-networks with multifunctionalities have been prepared from a single macromolecular platform to be used in various biomedical applications.

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Acknowledgments

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Polymer Therapeutics and Formulations for Applications in Brain Tumours

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Introduction

The prognosis for many patients with brain tumours is still very poor. The difficulty of delivering drugs across the Blood Brain Barrier (BBB) is an acknowledged problem, but there are also issues relating to heterogeneity of many tumours. We are interested in non-systemic routes for introducing drugs into the brain, and are developing formulations suitable for use as depots, following surgical resection of primary tumours, in order to counter residual cancer cells and recurrent disease. We are also exploiting the properties of a range of polymers to encapsulate different classes and combinations of drugs, with a focus on multi-functional systems that can be injected but which form gels in the post-resection cavity.

Experimental Methods

PEG-poly(caprolactone) and PEG-PLGA materials were prepared by ring-opening polymerisations, and poly(β-aminoester)s (PBAE) as described previously. Doxorubicin was conjugated to polymers via urea linkers while siRNA was complexed to protonated amine residues in the PBAE backbone. Glioblastoma and medulloblastoma cells were cultured according to existing protocols.

Results and Discussion

Treatment of cancer cell lines in 2D or 3D culture indicated that amphiphilic polymers were rapidly internalised (Figure 1). Cancer cell kill by cytotoxic drugs, and gene knockdown by specific siRNAs was also observed, with IC50 values for polymer-doxorubicin conjugates varying between 2-7 μM.

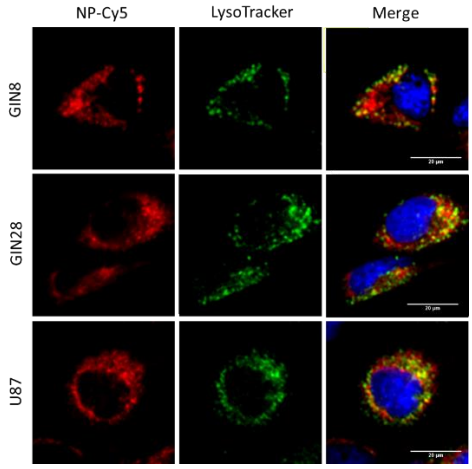

Figure 1. Cells treated with 50 μg/mL labelled polymers for 4 hours. Merged images show polymer-Cy5 signal (red), lysosome stain (green) and Hoechst 33342 (blue). Scale bar = 20 μm.

Conclusions

Our data so far show that the new formulations are effective in 2D and 3D in vitro, but efficacy in vivo will require greater temporal control of multi-drug release.

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Acknowledgments

This work was supported by the EPSRC [grant numbers; EP/N03371X/1; EP/H005625/1; EP/L013835/1; EP/L01646X/1], the Royal Society [WM150086] to CA and the Little Princess Trust/Children's Cancer and Leukaemia Group [grant number CCLGA 2019/32] to RR.

Therapeutic polymer systems based on self-assembly: from non-covalent to covalent

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Introduction

Polymeric materials are highly important in biomedical engineering. Self-assembly is widely used to fabricate biomedical polymer systems such as nanoparticles, vesicles, fibers and hydrogels. Current methods, however, rely on relatively weak non-covalent interactions, like hydrophobic and electrostatic interactions, which are sensitive to environmental conditions such as solvent polarity, temperature, ionic strength, pH, and co-solutes. Furthermore, controlling the architecture based on non-covalent self-assembly is difficult in many cases. We developed non-covalent self-assembly strategies to construct various polymeric systems to address unmet needs in clinical chemotherapy and immunotherapy[1]. We have engineered Π-Π stacked polymeric micelles for chemo-immunotherapy, vesicles based on polymer-prodrug conjugates for activating antigen presenting cell. Recently, we identified a new mechanism of polymer self-assembly which is triggered and controlled by covalent bonding for biomedical engineering purposes.

Experimental Methods

Our polymers were synthesized by controlled/free radical polymerization and prodrug chemistry. Polymeric micelles and nanovesicles were formulated by nano-precipitation. Covalent polymer self-assembly was performed in aqueous solutions by mixing polymers and the corresponding crosslinkers.

Results and Discussion

Amphiphilic polymers self-assembled into stable micelles which showed high stability in the blood circulation and efficient tumor accumulation after i.v. injection. Polymer conjugates of self-immolative TLR7/8 agonist prodrugs self-assembled into nanovesicles which were taken up by APCs and digested in endo/lysosomes to stimulate intracellular TLR[2]. This led to potent and durable immunoactivation *in vivo*. Covalently self-assembled polymers formed nano-to-macroscale polymer networks to deliver small molecule and macromolecular drugs for cancer therapy.

Conclusions

We employed conventional non-covalent self-assembly strategies and novel covalent self-assembly to construct materials ranging from nanoparticles to macroscale networks as therapeutic polymer systems.

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ROS-scavenging polymers for stealth-responsive conjugation

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Introduction

Here we show the potential of the combination of the concept of stealthness and responsiveness in view of the development of therapeutically active and stable (protein) conjugates.

With the aim to reduce interactions with biomolecules, 'stealth' polymers such as poly(ethylene glycol) (PEG) are conjugated with e.g. therapeutic proteins, reducing their immune recognition and its degradability, and thus prolonging their circulation time. There are growing concerns over PEG's own immunogenicity, potentially leading accelerated blood clearance (ABC). Further, classical 'stealth' polymers offer modest protection from denaturation during lyophilization or dry storage, and none from oxidation (by biological oxidants, Reactive Oxygen Species (ROS)).

In our group, we have long experience with ROS-responsive materials (organic polysulfides [1]), most typically used directly as therapeutic agents due to the anti-inflammatory effects of ROS removal [2].

Here, we illustrate the development of an 'active-stealth' platform, which is based on poly(thioglycidyl glycerol)(PTGG), a macromolecular scaffold that combines a) ROS scavenging, b) a polyol (sugar-mimetic) structure, c) (potentially site-specific) conjugability to biomolecules. The latter was exemplified using an enzyme (lysozyme) and an immunogenic protein (ovalbumin), and their size and grafting density-matched PEGylated derivatives.

Results and Discussion

In human plasma, PTGG is significantly less immunogenic than PEG (up to 35% less complement activation), but also features a potent ROS-scavenging and anti-inflammatory action (~50% reduction in TNF-α in LPS-stimulated macrophages at only 0.1 mg/mL). PTGG can also be efficiently conjugated to proteins via a one-pot processes; molar mass- and grafting density-matched PTGG-lysozyme conjugates demonstrated superiority to their PEG analogs in terms of both enzyme activity, and stability against freeze-drying or oxidation; the latter is due to sacrificial oxidation of methionine-mimetic PTGG chains. In rats, PTGG-ovalbumin (used as a model immunogenic protein) displayed a considerably longer circulation than PEG-ovalbumin (t1/2 = 28.2 vs 14.1 hours, 1.4-fold higher AUC on first dose) without an associated ABC effect (t1/2 = 26.3 vs 10.7, second dose).

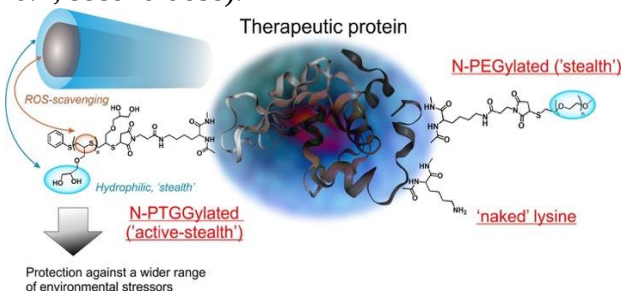

Figure 1. Comparative sketch of protein conjugation with active-stealth polysulfides (PTGG) in comparison to PEGylation.

Conclusions

PTGG's 'active-stealth' character makes it a promising alternative to PEG in bioconjugation.

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Derisking the GMP polymer supply chain for APIs and RNA encapsulation: A journey from R&D to routine commercial production

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Introduction

From a promising discovery in the lab to a solution that can reach the final intent to treat the patients, the journey is very long and most of the challenges to overcome unknown by the first developers.
During this presentation, the speakers will present the main steps of this journey in the context of GMP Polymers and share their experience on technical and scientific points to address through the way.

Results and Discussion

The presentation will address the issues to avoid when raising a project to a routine production in the case of GMP polymers while such products are entering in an increasing number of commercial drug formulations and medical devices. For instance, polymers as PLGA, PEG, PEI, polyaminoesters, polysaccharides, polyorthoesters are now commonly used as encapsulating, complexating, vectorization agents i.e. for API and nucleic acids.
Simultaneously, this category of drug substances used as functional excipients is subject to a strengthening regulatory and quality context, slowly reaching the level of expectations of an API.

Even a theoretical best-in-class polymer candidate developed in the lab will have to travel against several headwinds to reach a derisked routine production.
First, its manufacturing process has to be scaled from laboratory to industrial scale within a long and complex process, taking into account numerous considerations (HSE, product recovery and packaging, raw material sourcing etc...). Parallel to this step, all the regulatory and IP potential issues have to been anticipated

And when you reached a routine production (validated process with validated analytical methods, stability data, regulatory filings and compliance, proved efficiency and control of toxicity…) you still need to consider additional elements to ensure a reliable procurement : geographic and geopolitic strategy, sourcing of raw materials and equipment, regulatory monitoring…

Conclusions

The steps from an identified polymer candidate to an established GMP routine drug substance will be presented and discussed, showing the need to define strategy from early stage in order to reach cost-efficiency and shortest timelines and finally transform a project to a solution.

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PCAS SA, part of the SEQENS group

Polypeptide-Based Multivalent Nanoconjugates as Modulators of Tumor Microenvironment

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Introduction

The tumor microenvironment (TME) comprises non-cancerous stromal components such as the extracellular matrix (ECM), blood vessels, infiltrating immune cells, and various associated tissue-specific cells [1]. This unique environment, which emerges during tumor progression via complex interactions between host and tumorous tissue, has been proposed as a target for anti-tumor therapies [2]. Understanding the unique nature of the TME and implementing rational design by engineering biodegradable, multivalent polymeric nanocarriers (such as polypeptides [3]) may foster the development of more efficient anticancer nanotherapeutics.

Results and Discussion

Our studies demonstrated that polyglutamate-based polymers (PGA) represent excellent candidates for TME targeting due to their architectural versatility, biodegradability, and multivalency that allows the rational design of polymer-based combination therapies and the implementation of targeting strategies. We obtained PGAs through N-carboxyanhydride ring-opening polymerization (NCA-ROP) and introduced various functionalities through post-polymerization techniques to yield a set of orthogonal reactive attachment sites [4]. Following a bottom-up strategy, we obtained self-assembling star-based polypeptide architectures that formed supramolecular nanostructures with interesting properties [5], including a lymphotropic character highly suitable for nanovaccine development. This strategy, combined with adequate bioresponsive polymer-drug linker design [6] and drug selection and tumor-associated antigens or targeting moieties [7], allowed us to achieve proof-of-concept for metastatic breast cancer [6,7], melanoma, pancreatic cancer and castration-resistant prostate cancer treatment.

Conclusions

The rational design of polypeptide-based therapeutics, incorporating bioresponsive elements and targeting moieties for the TME, could significantly enhance their anticancer therapeutic efficiency. Adequate tropism and appropriate drug release kinetics represent crucial parameters for achieving an adequate safety:efficacy ratio and securing an adequate therapeutic window for future treatments.

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Role of Biomaterials in Endogenous Tissue Regeneration

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Introduction

Endogenous tissue engineering is applied to the process in which tissue heals in response to different stimuli. These stimuli can be retrieved by means of biomaterials/scaffolds and their properties, such as composition, structure, mechanical properties, and release of molecules.

The overall goal is to engineer novel solutions for cardiac healing based on acellular devices able to create a regenerative microenvironment that will lead to endogenous cardiac regeneration. We aim to work with polyesters to use degradation product, Lactate, to induce changes in the cell residing microenvironment, promoting the reprogramming of cardiomyocytes and cardiac fibroblasts in vivo, cell recruitment and vascularization. Lactate has been already identified as a molecule capable of inducing regeneration (1,2). In this work we analyze the effect of lactate on cardiac cells to stimulate endogenous cardiac regeneration.

Experimental Methods

We have tested different concentrations of lactate with postnatal cardiomyocytes, human cardiac derived induced pluripotent stem cells (CD-IPSc) and human cardiac fibroblasts. Langerdorff assays were used in adult mice hearts to evaluate the effect on adult tissue.

Proliferation and de-differentiation of cardiomyocytes were tested. Some materials were developed to match the needed lactate concentrations.

Results and Discussion

20mM Lactate enhanced mice cardiomyocytes proliferation and induced dedifferentiation, expressing genes related to cell reprogramming and proliferation.

CD-IPSc cultured with lactate have shown a response to lower concentrations (between 4-6 mM) and an effect on cell division was observed in a specific protein related to karyokinesis.

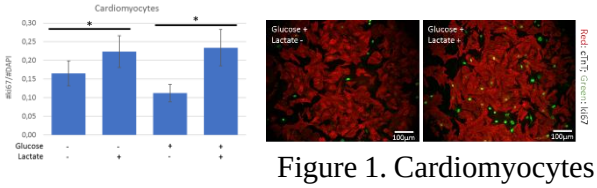

Figure 1. Cardiomyocytes from post-natal mice cultured on 20 mM lactate present proliferation activation, showing a) higher levels of ki67, b) observed by immunofluorescence

Exogenous lactate significantly reduces the expression of the myofibroblasts markers (protein and gene expression levels), it delays the characteristic migratory profile of activated fibroblasts, and it reduces the expression of various detrimental cytokines for cardiac repair.

Conclusions

Exogenous lactate appears to prevent cardiac harm and promote a proregenerative environment by stimulating cardiomyocytes proliferation and diminishing fibroblasts activation towards fibrosis. Altogether, this study further supports the potential use of lactate for in situ cardiac regeneration treatments.

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FROM BIOLOGICAL ORGANIZATION PRINCIPLES TO SUPRAMOLECULAR BIOFABRICATION

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Introduction

Living systems have evolved to grow and heal through "biological organization principles" (BOPs) capable of organizing molecular and cellular building-blocks at multiple size scales. These BOPs emerge from cooperative interactions and chemical networks between multiple components, which allow biological systems to diversify, respond, and optimize [1].

Experimental Methods

This talk will present our laboratory's efforts to combine supramolecular events found in nature such as self-assembly, disorder-to-order transitions, or diffusion-reaction processes with engineering principles and processes to design synthetic materials that exhibit properties of biological ones (Figure 1).

Results and Discussion

I will describe methodologies to develop: a) hydrogels for 3D cell culture with tuneable physical and chemical properties to recreate features of the tumour microenvironment [2], b) self-assembling fluidic devices [3], and c) hierarchically mineralizing materials [4,5].

Conclusions

The talk describes processes capable of generating materials with heterogenous, yet controlled, composition; tuneable mechanical properties including soft environments and tailored molecular diffusion capabilities; and different types of multicellular structures.

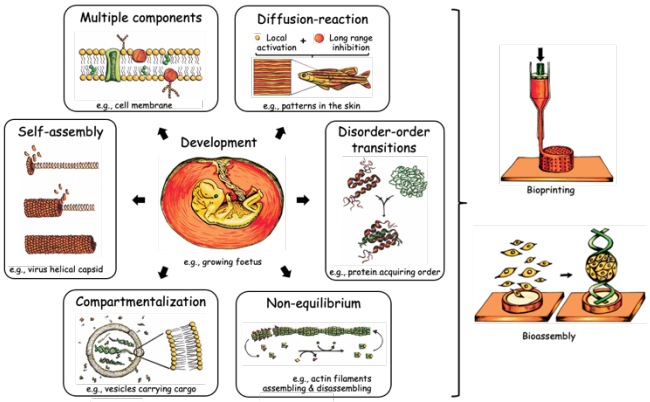

Fig 1. Vision for a supramolecular biofabrication toolkit integrating biological organization principles with fabrication techniques.

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On the Interaction between Polymers and Light: From Chemical Design towards Medical Device

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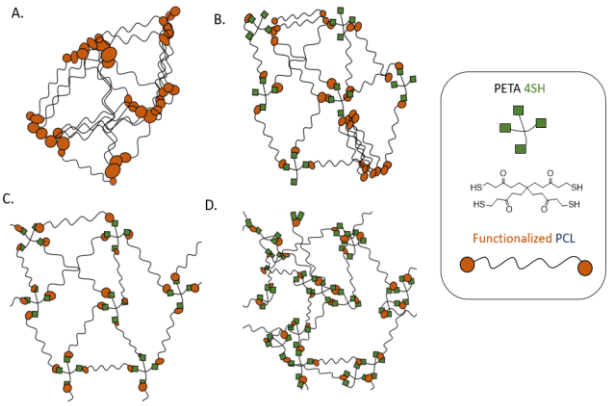
Biofabrication is a specific area within the field of tissue engineering which takes advantage of rapid manufacturing (RM) techniques to generate 3D structures which mimic the natural extracellular matrix (ECM). A popular material in this respect is gelatin, as it is a cost-effective collagen derivative, which is the major constituent of the natural ECM. The material is characterized by an upper critical solution temperature making the material soluble at physiological conditions. To tackle this problem, the present work focusses on different gelatin functionalization strategies which enable covalent stabilization of 3D gelatin structures [1-4]. In a second part, synthetic acrylate-endcapped, urethane-based precursors (AUP) based on polyesters, will be discussed with exceptional crosslinking behaviour and CAD-CAM mimicry compared to conventional materials [5, 6]. Within this synthetic material class, also insight will be provided on the shape memory properties of polyester-based AUPs [7]. Both chain growth and step growth polymerization mechanisms (see figure) along with their mechanical properties and processability potential will be addressed [5, 6]. Several polymer processing techniques will be covered including conventional 3D printing using the Bioscaffolder 3.1, Digital Light Projection (DLP) [6] and two-photon polymerization [2, 3, 5]. A number of biomedical applications will be tackled including adipose tissue engineering, vascularization [2], ocular applications, etc. In a final part, attention will be paid to the valorization of our biomaterial platform technology through the launch of our spin-off company BIO INX. The results show that chemistry is a valuable tool to tailor the properties of (bio)polymers towards light-based processing while preserving the material biocompatibility.



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The Third Strategy in Tissue Engineering Enabled by High Resolution 3D Printing

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Introduction

Current approaches in Tissue Engineering (**TE**) can be roughly categorized into scaffold-based and scaffold-free. Recently a third TE strategy, combining the advantages of these seemingly opposing approaches, while circumventing their drawbacks was proposed [1]. This novel strategy is based on self-assembly of tissue units (**TU**s) consisting of microscaffolds containing cell spheroids. Realization of such microscaffolds is enabled by high-resolution 3D printing and the recent availability of suitable materials [2, 3].

Experimental Methods

Two-photon polymerization (2PP) was utilized to fabricate highly-porous microscaffolds from a commercially available DEGRAD INX X100 photopolymer (BIO INX BV). Immortalized human adipose-derived mesenchymal stem cells (hASC, ASC/TERT1 Evercyte) were expanded in fully supplemented EGM-2 with 10% serum. Each buckyball (**BB**) microscaffold (diameter of 300 µm), residing in an antiadhesive well plate, was seeded with 4000 hASCs in order to form spheroids (see Fig. 1a). Differentiation towards osteogenic and chondrogenic lineages achieved by culturing TUs in according media for 21 days.

Results and Discussion

It was demonstrated that hASCs rapidly form spheroids directly within the microscaffolds. The resulting TUs maintained high viability and preserved their chondrogenic and osteogenic potential. Multiple TUs were successfully merged to form larger constructs, which offer a great perspective to fill up tissue defects [4].

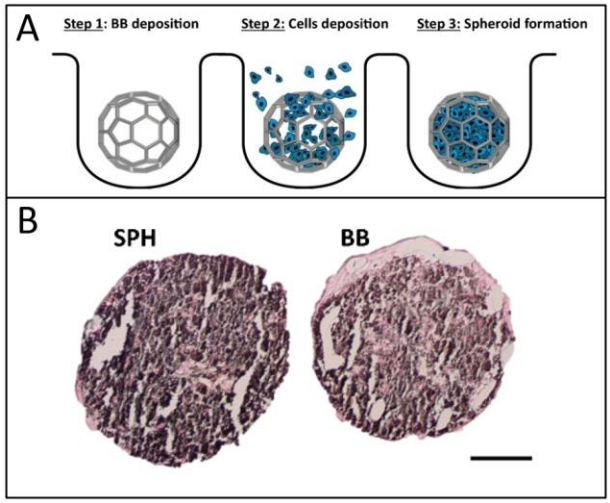

Figure 1. Top: Schematics of spheroid formation directly within a buckyball (BB) microscaffold. Bottom: Osteogenic potential - comparison of mineral deposition (Von Kossa) in regular spheroids (SPH) and BBs (scale bar is 100 µm).

Conclusions

The microscaffolds carrying high density of cells are promising building blocks for cartilage and bone TE facilitated by bottom-up assembly.

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Inverse shape-memory effect in hydrogels

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Introduction

The shape-memory effect of polymers has been used in the design of medical devices for minimally-invasive surgery [1]. When inserted in the body, compressed porous implants can expand upon heating from ambient to body temperature for instance to close a blood vessel. A surgical suture can form a preprogrammed knot or they self-tighten. The shape-changing behavior is a response to heat and characterized by occurring one time and being unidirectional. The effect cannot be reverted by cooling. While shifting their shape, such polymers soften. This feature is also relevant for biomedical applications. Increasing the elasticity of a smart suture can support wound healing in soft tissues by adapting the closure device to the space requirements of healing tissue. In contrast to implants providing structural support, tissue reconstruction faces an unmet need for soft active scaffolds capable to substitute the extracellular matrix temporarily. Their softness and elastic behavior make hydrogels promising candidate biomaterials for such purpose. Polymer networks from hydrophilic network chains have been equipped with hydrophobic, crystallizable side chains. These form hydrophobic phases serving as thermally controlled switches for a classical heat-induced shape-memory effect.[2] An essential design criterium of shape-memory hydrogels is their porous morphology. This architecture of the shape morphing structure pertains its macroscopic dimensions. Here swelling only occurs on the pore wall level, whereby typically pore diameters are in the μm scale.

Results and Discussion

Here a microporous hydrogel is introduced, which performs a shape shift in response to cooling. Such inverse shape-memory effect shall be triggered within a temperature range, which is compatible with physiological environments. An additional feature, which shall be achieved with such soft scaffolds, is a physical biofunctionalization process by loading the structure with platelet-rich plasma (PRP) eibiting high bioactivity. These requirements were fulfilled by a polymer network with oligo(ethylene glycol)-oligo(propylene glycol)-oligo(ethylene glycol) chain segments. These triblock copolymer segments form micelles in the hydrogel structure when heated to 37 °C. Pore walls are compacting while the average pore diameter increases. On the macroscopic level, only a volumetric shrinkage of up to 12% could be determined. The increased G-modulus at high temperature allows to fix a temporary, macroscopic shape. The original shape can be almost completely recovered by cooling to 5 °C. At the same time the pore diameter decreases and the hydrogels softens. The swelling of the hydrogel pore walls, its interconnected pore morphology and the change in the spatial organization of the network segment, support an effective loading with PRP.

Conclusion

An inverse shape-memory effect could be successfully implemented in a microporous hydrogel, which can shift its shape upon cooling.

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Living polymerisation of water-soluble monomers towards the fabrication of soft cellular scaffolds

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Controlled/living radical polymerisation techniques, including reversible-addition fragmentation chain-transfer (RAFT), have transformed the field of polymer chemistry in the last few decades, affording the production of polymers with precise control over molecular weights, dispersities, and architectures.[1] More recently, Boyer and co-workers reported a robust and highly efficient photo-induced living polymerization, which was set to change the radical polymerisation landscape.[2] In their work, a photoredox catalyst was employed to generate an excited species under irradiation, which was then able to reduce thiocarbonylthio compounds (RAFT agents) *via* photoinduced electron transfer (PET), initiating polymerization of monomers. This technique presents several merits in comparison to the conventional RAFT mechanism: the polymerization reactions can be performed at room temperature, in the presence of air, using low energy blue light in tandem with catalyst doses in the ppm range. Following this successful discovery, Hawker and co-workers reported the use of PET-RAFT to polymerise monomers directly at the cell surface, decorating the cell membrane with a poly(ethylene glycol) analogue.[3] More recently, Bradley and co-workers described the first ever attempt of polymerisation inside living cells using *N*-(2-hydroxypropyl) methacrylamide (HPMA) and sodium 4-styrenesulfonate (NaSS) as monomers.[4] Inspired by these exciting advances, we aim to design new cytocompatible materials using water-soluble precursors that can be polymerised through RAFT and PET-RAFT methodologies. The new materials would also be capable of undergoing post-polymerisation modifications directly in water and can be used as cell scaffolds for applications in the tissue engineering remit.

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Nanogels as a versatile multi-modal biomedical nanomaterial

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Introduction

Nanogels are hydrogel-based nanoparticles that are highly tunable in chemical composition and physicochemical properties.[1] These nanoparticles are highly versatile in their uses and allow for several functions to be combined including antimicrobial properties, fluorescence and MRI tracking and imaging, anti-adhesive, controlled release, and responsiveness to various stimuli.[1] Because of this variety of functions and properties, the particles are used in controlled delivery, imaging, theranostic approaches and functional biomedical multi-modal coatings.[1-5] The studies presented provide insights in the capabilities of such nanogels, the preparation, modification, and the use of them together with biological systems. The ease of scaling, the diversity, and ease of applicability make these particles very powerful.

Results and Discussion

The approach for nanogel formation is by precipitation polymerization, which allows for co-polymerizations as well as control over location of the monomers.[5] Via sequential addition of monomers to the reaction mixture, core-shell nanogels can be formed allowing more versatility for implementing function. Post-modifications allow for inclusion of medically relevant attributes such as antimicrobial properties via e.g. quaternization[3,4], inclusion of MRI tracers via peptide coupling or one can already introduce such function by using modified monomers bearing such attributed[5]. By precisely controlling the physicochemical properties such as charge or stiffness, one can utilise it not only as a carrier (Fig. 1) but also control the interactions with its surrounding that allows strong and easy applicable coatings (Fig. 1) using surface activation and electrostatic interactions.

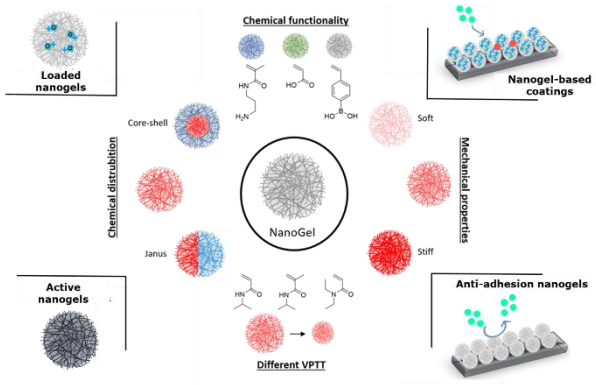

Figure 1. A versatile approach using nanogels for suspension and coating applications.

Conclusion

Nanogels offer tremendous possibilities to be used as functional suspension-based structures and coatings. These will complement the biomedical field with the already more established inorganic or liposome-based nanoparticles.

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Fabrication of a 3D in vitro model of the human gut microbiota

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Introduction

The word "microbiota" refers to all the microorganisms that reside within human body. An alteration of the human gut microbiota composition seems to have one of the leading roles in the development of several systemic diseases ranging from dementia to autism, from renal (e.g. Pyelonephritis) to hepatic diseases (Non-alcoholic fatty liver disease).

Here, we present a novel approach to the culture of the human gut microbiota using an electrospun scaffold to support the adhesion and proliferation of the microorganisms.

Experimental Methods

We tested natural (i.e., gelatin [1]) and synthetic (i.e., polycaprolactone (PCL)) polymers to fabricate two different electrospun structures. Both were coated with a solution of mucins to change the chemical features of their surface, to identify the optimal environment for developing a three-dimensional in vitro model of the human gut microbiota. To assess the adhesion and microbial proliferation on the electrospun structures, crystal violet biofilm assays and imaging acquisitions were carried out.

Results and Discussion

These data indicate that electrospun gelatin structures in the absence of a mucin coating are more suitable than the other structures used in this work in supporting the adhesion and growth of the fecal microbiota for prolonged times of in vitro culture, thus suggesting their supportive role, especially in the later stages of biofilm formation (as stated also from the adhesion assay with crystal violet in Figure 1 with a better adhesion at 7 days).

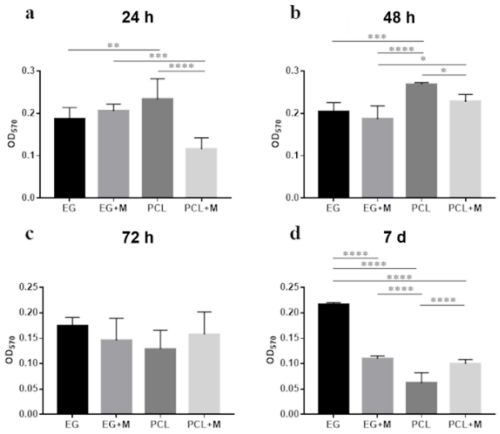

Figure 1. Analysis of the microbial biofilm formation using crystal violet quantification of the fecal microbiota.

Conclusions

This work may represent a first step toward the generation of a reliable in vitro model of the human gut microbiota. In the future, this in vitro model could be exploited to obtain more complex systems, i.e., including human cells, to study the intricate relationship between microorganisms and the human host.

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Thermosensitive Shrinking Hydrogels for High Resolution 3D-Printing

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Introduction

Printing high resolution scaffolds with intricate geometries and small sizes (< 100 µm) from soft hydrogels is not only a challenge, but also of high interest in biofabrication for the regeneration of complex tissues. Hydrogel shrinking techniques have recently been proposed for 3D printing of scaffolds to enhance the resolution of the structures post-printing. For that purpose, we recently developed a shrinking technology based on water expulsion upon complexation of charged polymers.[1]
In this study, we designed an alternative shrinking technology based on a temperature trigger. This thermosensitive shrinking method for printed hydrogels makes use of the lower critical solution temperature (LCST) behavior of poly *N*-isopropyl acrylamide (pNIPAM).

Experimental Methods

Biopolymers, gelatin and silk fibroin, were functionalized with methacryloyl moieties resulting in GelMA and SilkMA, respectively. The dissolved biopolymers were mixed with NIPAM and subsequently printed. Upon exposure to light, a crosslinked network was formed and hydrogel shrinking behavior was evaluated upon heating above the LCST.

Results and Discussion

Both GelMA/NIPAM and SilkMA/NIPAM hydrogels structures reduced in volume by a factor 2 when heated to 37 °C. This shrinking effect was found to be fully reversible when samples were cooled back below the LCST. After shrinking, both types of hydrogels showed an increase in the gel strength and an increase in the Young's modulus. Both volume reductions and cell viability of conditionally immortalized proximal tubule cells showed a correlation with increasing ratios of NIPAM to methacroylated polymer concentrations while keeping the total polymer concentrations constant.
These hydrogels were applied to volumetric printing to obtain 3D constructs with complex geometries capable of reducing their size by a simple temperature increase to 37 °C. Both positive and negative features were printed with a resolution enhancement of a factor 2-3 upon temperature induced shrinking.

Conclusions

This study provides a new technology to reach resolution enhancement by shrinking of hydrogels, which in combination with the versatility of these materials shows their potential for tissue engineering applications.

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Acknowledgments

The NWO/VICI (no. 18673) programme is acknowledged for funding.

2D- and 3D-printing of aerogels for biomedical applications

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Introduction

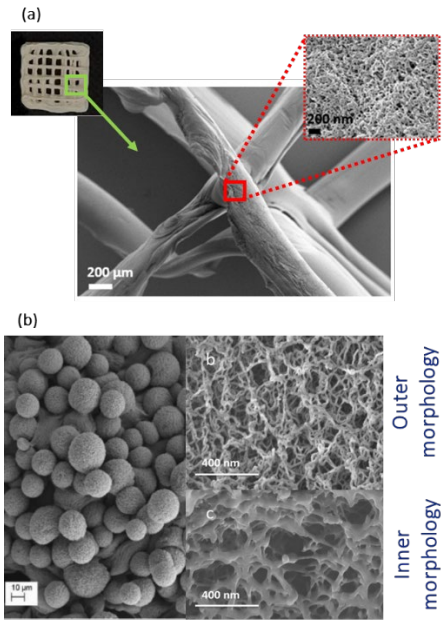
Aerogels are nanostructured materials consisting on solid and open mesoporous networks, endowed with high specific surface areas and porosities. The extracellular matrix-mimicking nanostructure of aerogels are especially attractive as scaffolds for regenerative medicine. Aerogels are also especially advantageous as carriers of bioactive compounds. In this work, the technological combination of 2D- & 3D-printing and aerogel technologies is evaluated to obtain advanced materials for two biomedical applications: pulmonary drug delivery[1] and personalized scaffolds[2].

Experimental Methods

Alginate gels are obtained by inkjet 2D-printing and extrusion-based 3D-printing following an ionic (Ca^{2+}) gelation mechanism. Gels were dried using supercritical CO_2 (120 bar, 40ºC, 5 g/min) for 4 h. The obtained aerogels in the form of microspheres and of personalized CAD 3D-designs were evaluated regarding their physicochemical and biological properties.

Results and Discussion

Two innovative aerogel solutions were obtained by material design control in the 2D (particle diameter) and 3D (CAD-patterns) dimensions. The dual-porous aerogel scaffolds obtained by 3D-printing were bioactive, biocompatible and with personalized shape (Fig. 1a). On the other hand, aerogel inkjet printing resulted in porous microspherical drug carriers (Fig. 1b) with excellent reproducibility, narrow particle size distribution (24±4µm) and suitable release profiles for pulmonary delivery.



Conclusions

Drug products and medical devices with advanced performances were produced by combined aerogel and printing technologies. The obtained aerogel structures show superior properties for pulmonary drug delivery and personalized regenerative medicine.

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Acknowledgments

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Hybrid hydrogels based on natural and synthetic polymers

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There is great need for novel degradable hydrogels that stimulate the regeneration of non-functioning, diseased or damaged tissues, and many natural and synthetic polymers have already been used for this.

In general, the physicochemical properties of synthetic polymers (e.g. mechanical properties, degradability), can be tuned for a certain application. It remains a challenge, however, to provide synthetic polymers with biological properties like cell-adhesive sites or cell-instructive properties. Natural polymers like extracellular matrix proteins and polysaccharides possess biological information, which is essential to cell adhesion and directing tissue renewal and deposition.

Combining the advantageous properties of both classes of polymeric biomaterials into hybrid hydrogels allows the preparation of new tissue-compatible biomaterials with suitable mechanical properties for the active regeneration of tissues.

Here we show that after methacrylate-functionalization a variety of natural- and synthetic polymers can be combined to form hybrid hydrogels that possess enhanced features over the single networks in terms of their mechanical and biological properties.

Biomimetic polymers as smart functional therapeutics

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Introduction

Our scientific approach is based on biomimicry, as we engineer synthetic mimics of natural macromolecules (such as proteins or glycoproteins), and explore their controlled and tunable self-assembly to form self-assembled structures similar to those found in nature (such as virus or cell membranes). In this context, we develop for instance polymer-based self-assembled nanoparticles, mostly polymeric vesicles, also named polymersomes, with high loading content of active pharmaceutical ingredients (e.g., anticancer drugs, peptides, proteins), multimodal imaging agents (e.g., MRI probes, NIR dyes) and targeting ability.

Experimental Methods

Our expertise includes the synthesis of precise, biocompatible polymers such as polypeptides (by chemical synthesis or recombinant DNA technology), polysaccharides, and polypeptide-polysaccharide conjugates.

Results and Discussion

We present here an overview of the self-assembly of amphiphilic block copolymers developed in our laboratory, focusing polymersomes, and their contribution in nanomedicine. We pay particular attention to block copolymer vesicles based on polysaccharides, polypeptides and proteins. In particular, we have recently developed synthetic strategies for the design of glycosylated polypeptides and polysaccharide-polypeptide biohybrids with controlled placement of the sugar functionality. We have been particularly interested in the design of amphiphilic copolymers capable of self-assembling into well-defined micelles and vesicles that can advantageously be loaded with drugs and present a surface with multivalent presentation of bioactive saccharide moieties. Recent developments at the interface of bioengineering and polymer science, based on elastin-like polypeptides, relevant for regenerative medicine, glycoproteins and lipoprotein mimetics, will also be proposed. Finally, our most recent advances in the design of complex, compartmentalized and functional artificial cells will be presented. Such a system is a first step towards the challenge of structural cell mimicry and functionality, and could act in the future as an autonomous artificial cell capable of detecting and healing in situ any biological deregulation

Conclusions

Our goal is to push forward such systems to the clinics, while pursuing our efforts in addressing fundamental questions of nanoparticle self-assembly and membrane properties.

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Bio-aerogels : prospects for biomedical applications

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Introduction

Porous polysaccharide materials have been used in biomedical and pharmaceutical application since long time. Aerogels are low-density nanostructured porous materials with high specific surface area. In our group we are developing bio-aerogels based on polysaccharides. In this presentation the main ways of bio-aerogels' preparation, shaping and properties will be presented; examples of using them in biomedical applications will be given and discussed.

Experimental Methods

We prepare aerogels via polysaccharide dissolution, gelation (in some cases this step can be omitted), solvent exchange and drying with supercritical CO_2. We use cellulose, starch, pectin, chitosan and hyaluronic acid and we make aerogels in the shape of monoliths or beads.

Aerogels are characterized by their density, morphology with scanning electron microscopy, specific surface area with nitrogen adsorption and mechanical properties.

Results and Discussion

The examples of bio-aerogel in the shape of a monolith (disc of diameter around 3 cm) and beads (diameter of several hundreds of microns up to few millimeters or of few tens of microns) are shown in Figure 1 together with aerogel morphology (here, for pectin aerogel). Density (around 0.05 – 0.2 g/cm^3) and specific surface area (200 – 600 m^2/g) of bio-aerogels depend on the type of the polymer and can be tuned by varying processing conditions. Aerogel behavior in physiological conditions (for example, for the release of drug) depends by polysaccharide solubility (swelling and/or dissolution or not). Different examples of the potential use of bio-aerogels and their composites as drug delivery matrix will be given.

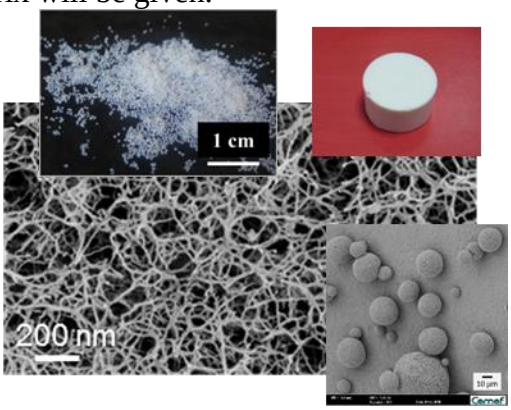

Figure 1. Examples of bio-aerogels in different shapes and of aerogel internal microstructure.

Conclusions

Bio-aerogels are based on neat polysaccharides with no crosslinker or toxic compound used for their preparation; they are highly porous materials with high internal surface area. They are thus potentially very attractive for various bio-medical applications such as wound dressings and/or drug carriers.

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Acknowledgements

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Poster abstracts

Poster number, presenting author and title

1. Hélène van den Berghe - Biodegradable PLA-PEU-PLA membranes: A new solution for the reduction of peritendinous adhesions
2. Francois Toussaint - PVP as PEG alternative for lipoplex modification in siRNA delivery application
3. Daniel Aguilera - Solubility and dissolution of hyaluronic acid in water
4. Lorena Infante - ROS-scavenging polysulfide nanoparticles display potent anti-fibrotic and anti-scar properties through inhibition of the fibroblast-to-myofibroblast transition
5. Coraline Chartier - Release of ascorbic acid 2-phosphate and dexamethasone phosphate from chitosan aerogels and cryogels in view of potential wound dressing applications
6. Feifei Ng - BEPO®: A bioresorbable polymeric in situ forming depot for the tunable sustained release of active pharmaceutical ingredients
7. Laurianne Legay - Hyaluronic acid aerogels prepared via freeze-thaw induced gelation
8. Giulia Coradello - Colloidal systems of sulfur(II)-based copolymers for anti-inflammatory therapies
9. Matilde Grosjean - Dual-crosslinked degradable elastomer with self-healing properties
10. Marion Négrier - Preparation, characterization and cytotoxicity evaluation of cellulose aerogels beads made using ionic liquid
11. Bas van Bochove - In vitro and in vivo degradation of photo-crosslinked poly(trimethylene carbonate-co-ε-caprolactone) networks
12. Anna Mitzakoff - Development of a 3D-printable polypeptide gel for bone regeneration
13. Nazely Diban - Graphene/polyacrylonitrile membranes induce spontaneous breast cancer cell spheroid formation
14. Marc Ankoné - Poly(1,5-dioxepan-2-one) (PDXO) networks prepared by photocrosslinking
15. Zeynab Mirzaei - Development of novel hybrid hydrogels based on chitosan/poly(2-oxazoline)s-block-poly(2-oxazine)s for bio-based aerogels fabrication
16. André Poot - 3D Printing of Porous PCL-b-PTMC-b-PCL and Nano-Apatite Composite Structures
17. Kasper Dienel - Additive Manufacturing of Patient-Specific Mandibular Bioimplants
18. Erik Hebels - The Covalent Entrapment of Fragile Compounds into Biodegradable Polymeric Micelles via a Native Chemical Ligation Crosslinker
19. Isabella Laurén - Antiviral activity of quaternized chitosan derivatives: structure-activity relationships

20. Maria Carracedo-Pérez - Sterilization of bio-based aerogels by supercritical CO2 technology
21. Coline Pinese - Design of hybrid polymer/peptide nanofibers for soft tissues regeneration
22. Lea Dejob - 3D printing of textile-like biodegradable membranes: effects of printing parameters on mechanical and structural properties
23. Barbara Blanco-Fernandez - Decellularized mammary gland bioinks for the development of 3D breast cancer models

Biodegradable PLA-PEU-PLA membranes: A new solution for the reduction of peritendinous adhesions

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Introduction

Peritendinous adhesions are complications known to occur after surgery and cause chronic pain and disability. Anti-adhesive biodegradable membranes, based on a new poly (lactic acid)-poly (ether urethane)-poly (lactic acid) (PLA-PEU-PLA) copolymer, was designed to be easily applied as barrier during tendon surgery to effectively prevent adhesions.[1] A preliminary *in vivo* study in a rat model of peritendinous adhesions was conducted to evaluate the membranes' degradation rate, tendon healing and anti-adhesion effect.[2]

Experimental Methods

PLA-PEU-PLA membranes were characterized by 1H-NMR, SEC, DSC and SEM. *In vivo* studies included macroscopic, histological, biomechanical and degradation evaluations.

Results and Discussion

PLA-PEU-PLA membranes were characterized in terms of structure, thermal and morphological properties. These membranes were flexible, easy to handle and adapted to clinical settings (Fig. 1).

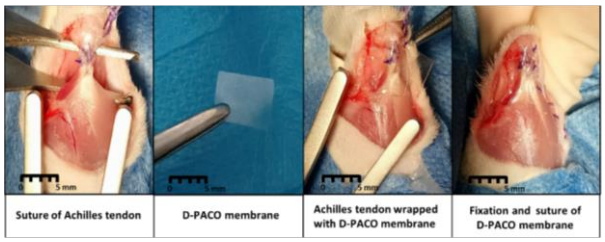

Figure 1. Flexibility and suturability of PLA-PEU-PLA membranes.

Macroscopic and histological evaluations of membranes showed anti-adhesion efficacy in a rat Achilles tendon model after 2 weeks' surgery and a reduction of the adhesions after 10 weeks. Histological and biomechanical results showed that membranes do not interfere with tendon healing. Moreover, *in vivo* degradation study by SEC showed that membranes filmogenicity was maintained at 2 weeks, whereas they were significantly degraded after 10 weeks.

Conclusions

The results showed that PLA-PEU-PLA membranes are adapted to clinical settings, they could effectively reduce peritendinous adhesions *in vivo* and promote tendon healing. The study also highlights the need to increase the experimental period and the sample size for further experiments as adhesions are still forming up to 10 weeks.

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PVP as PEG alternative for lipoplex modification in siRNA delivery application.

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Introduction

Liposomes are carriers of choice for numerous drug delivery applications.[1] The latter allows to incorporate a wide variety of hydrophilic and lipophilic drugs, to preserve the stability of these compounds and reduce the exposure of the organs to potentially toxic drugs. In particular, siRNA-loaded lipoplexes, resulting from the electrostatic interactions between the polar headgroups of cationic phospholipids and the phosphate groups of siRNA, are promising drug delivery vehicles for cancer therapy.[2] Pegylation of lipoplexes is a common strategy to prevent their rapid elimination by macrophages and prolong their circulation time in blood.[3] Nevertheless, pegylated liposomes/lipoplexes have some limitations as PEG may cause immunological responses as well as Accelerated Blood Clearance (ABC effect), which induces their recognition and elimination from the body.[3] As alternative to PEG, herein, we explore the use of novel poly(*N*-vinylpyrrolidone) (PVP) derivatives for the modification of siRNA-loaded lipoplexes (Figure 1).

Results and Discussion

A series of well-defined amphiphilic PVP-based polymers composed of a single or double aliphatic chain were synthesized by Reversible Addition Fragmentation chain Transfer (RAFT). The impact of the nature of the hydrophobic group and of the PVP molar mass on their insertion within lipid bilayers was studied by quartz crystal microbalance with dissipation monitoring (QCM-D). Dynamic light scattering and zeta potential analyses of the post-modified lipoplexes confirmed the ability of some PVP derivatives to incorporate within the membrane and to shield the positive charges of the lipoplexes. Additional tests also demonstrated the capacity of these innovative lipoplex modifiers to prevent protein adsorption.

In the future, *in vitro* and *in vivo* tests will be performed to further assess the relevance of PVP as alternative to PEG for the modification of siRNA-lipoplexes.

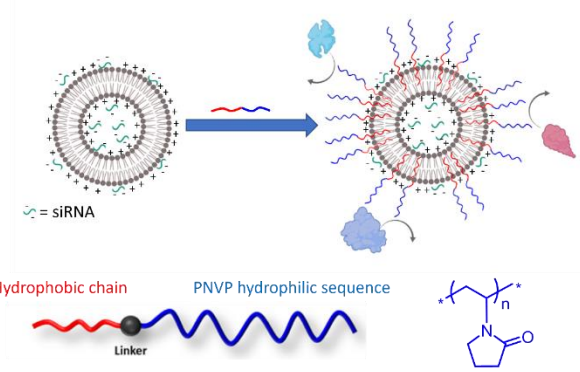

Figure 1. Amphiphilic PVP as lipoplex modifier for siRNA delivery.

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Solubility and dissolution of hyaluronic acid in water

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Introduction

Hyaluronic acid (HA) is a natural anionic polysaccharide heavily researched for its current and potential applications in biomedical field. Nevertheless, fundamental knowledge of its solubility and dissolution kinetics in water are missing. Quantitative nuclear magnetic resonance (NMR) and capillary electrophoresis (CE) can be used for determining polysaccharides solubility and monitor dissolution online[1,2]. A better understanding of the extent of HA dissolution in aqueous media could help improving current HA functionalization strategies for functional materials in medicine.

Experimental Methods

Samples of different molecular weights of neat HA were dissolved in D_2O for NMR and ultra-pure water for CE (HA 1 g/L). The solubility of HA was determined by ^{1}H NMR. Standard kinetic experiments were started immediately after HA addition.

Results and Discussion

The figure 1 shows two examples of HA dissolution monitoring in water by ^{1}H NMR and CE. The ^{1}H NMR experiments (Fig. 1a) indicated HA dissolution was not complete even after a week. The extent of HA dissolution is highly dispersed indicating heterogeneity of HA samples and it can be associated with a possible formation of nanoclusters. Thanks to the monitoring by CE (Fig. 1b) it was possible to demonstrate that the HA solution reaches a plateau after 12 h.

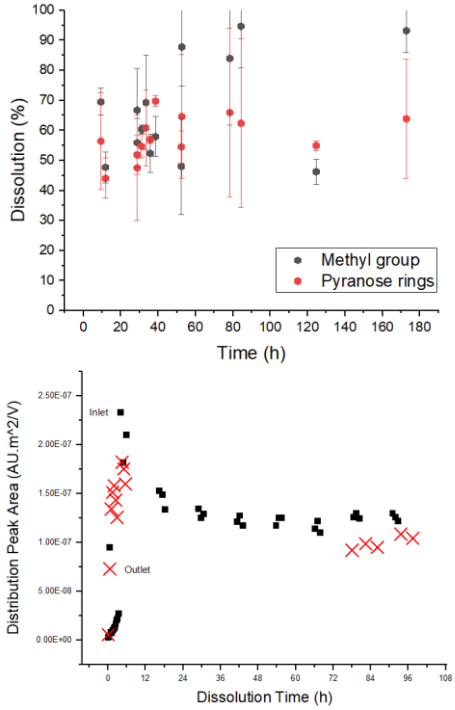

Figure 1. Examples of HA dissolution online monitoring a) by ^{1}H NMR spectroscopy c) by CE

Conclusions

The HA solubility, at maximum dissolution, was determined using quantitative ^{1}H NMR. HA solubility is around 60%, indicating than even if the 'solution' is completely transparent the polysaccharide was not fully dissolved. CE experiments indicated that the maximum dissolution of HA take places in less than 12 h.

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ROS-scavenging polysulfide nanoparticles display potent anti-fibrotic and anti-scar properties through inhibition of the fibroblast-to-myofibroblast transition

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Introduction

The fibroblast–myofibroblast differentiation is a crucial process in many physiological and pathological phenomena, including wound healing, fibrosis and cancer.[1] Although myofibroblast contraction is critical for wound healing, hypertrophic scaring is a pathological example of its dysregulation. Reactive Oxygen Species (ROS) play a critical role in the fibrotic pathogenesis of tissues where they act as secondary messengers in the TGFβ pathway. Antioxidant therapies are therefore an interesting possibility to block fibrosis and scaring. In this vein, polysulfides have been found to be excellent ROS-scavengers with potent anti-inflammatory effects.[2] We here have evaluated the ROS-scavenging effect of polysulfides nanoparticles (NP) and their ability to augment the fibrotic pathology.

Experimental Methods

PPS NP was synthetized by the ring-opening emulsion polymerization of propylene sulfide, in a waterborne medium stabilized by Pluronic F127, followed by crosslinking with pentaerythritol tetraacrylate. Human Dermal Fibroblasts (HDFa) were incubated in DMEM (10% FBS, 1% antibiotic solution, 1% L-glutamine). Cells were treated with PPS NPs, enzymes or additives in SFM or 10% FBS. Apoptosis and cell death were measured by Flow Cytometry analysis. Nanoparticle uptake and internalization were measured through the fluorescence signal of fluorophore-tagged NPs on cell lysates. Marker expression was analysed by mRNA signal through RT-qPCR analysis.

Results and Discussion

ED-A fibronectin is upregulated in response to ROS production (A). Treatment with PPS NPs or catalase but not superoxide dismutase can reverse the overexpression. TGFβ1-induced overexpression of α-SMA is both prevented and reversed by treatment with PPS-NPs, whereas treatment with catalase only marginally reduces α-SMA (B).

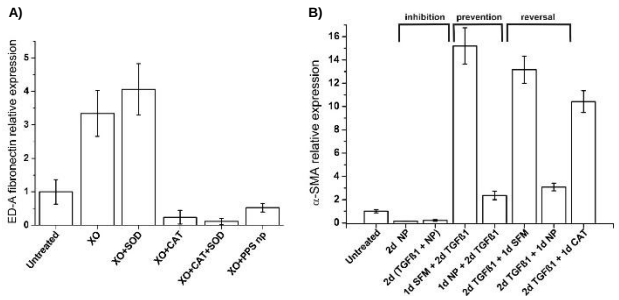

Figure 1. Expression of ED-A fibronectin and α-SMA mRNA in response to different pro-inflammatory stimuli and ROS scavenging.

Conclusions

This study represents the use of polymeric nanoparticles as non-conventional nano-antioxidant for the inhibition and reversal of myofibroblast differentiation in vitro. These results could have a significant impact in the design of novel antifibrotic therapies based on the scavenging of H_2O_2.

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Release of ascorbic acid 2-phosphate and dexamethasone phosphate from chitosan aerogels and cryogels in view of potential wound dressing applications

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Introduction

The biocompatibility as well as the antibacterial and antifungal properties of chitosan combined with the high specific surface area and the controlled porosity of aerogels[1] are very attractive in biomedical applications such as wound healing, tissue engineering and controlled drug release[2]. In this work, we investigate the *in vitro* release kinetics of ascorbic acid 2-phosphate (AAP) and dexamethasone phosphate (DEXP) from chitosan aerogels and cryogels as a function of process parameters.

Experimental Methods

Chitosan aerogels were prepared *via* dissolution, non-solvent induced phase separation, solvent exchange and drying with supercritical CO_2. Cryogels obtained by freeze drying were prepared from the same aerogel precursor for comparison. No crosslinker was used in both processes. Drug was loaded *via* impregnation into chitosan aerogel and cryogel precursor through diffusion. The loading efficiency was defined as the actual drug dose over the theoretical maximum drug dose in the gel. The kinetic of AAP and DEXP release was monitored *via* UV spectroscopy and UPLC, respectively, as a function of chitosan concentration, type of drying and pH of the release medium.

Results and Discussion

Chitosan aerogels and cryogels were prepared with a density of 0.11 and 0.06 g/cm^3, and 261 and 51 m²/g specific surface area, respectively[3]. Interestingly, the loading efficiency of AAP was higher than 100%, *i.e.,* between 300 % to 500 % showing a preferential interaction between chitosan and AAP. Higher was the material density and lower was the pH of the release bath (7.4 *vs* 8.9), slower was the release. Overall, half of AAP was released in 30 min to 3 h from cryogels and aerogels, respectively. Full release of AAP from aerogels was achieved in 50 to 80 h. Various models were tested to describe release kinetics.

Conclusions

Tunable properties of chitosan aerogels and cryogels allow controlling the kinetics of drug release, making these materials promising for controlled drug release.

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Acknowledgments

The project is performed within the CNRS "Thèse transverse" program. We thank Pierre Ilbizian and Julien Jaxel (PERSEE, MINES Paris) for drying with supercritical CO_2, Suzanne Jacomet (CEMEF, MINES Paris) for help in SEM imaging and PHC Van Gogh programme for funding.

BEPO®: A bioresorbable polymeric *in situ* forming depot for the tunable sustained release of active pharmaceutical ingredients

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Introduction: MedinCell is a clinical-stage pharmaceutical company that develops a portfolio of long-acting injectable (LAI) products for the sustained release of active pharmaceutical ingredients (APIs) using its proprietary technology, BEPO® [1].
BEPO® is composed of a blend of diblock (DB) and triblock (TB) amphiphilic PEG-PLA copolymers that are dissolved in a biocompatible solvent, together with an API, yielding injectable solutions or suspensions. Upon injection into an aqueous environment, such as the subcutaneous tissue, the solvent diffuses out allowing for the precipitation of the copolymers: this results in the formation of a polymeric depot that physically entraps the API. The subsequent drug release is then driven by API diffusion and polymer degradation [2].
Current efforts within the Research Department aim at deepening the understanding about the formation and resorption of the depots through comprehensive degradation studies [3], as well as improving and expanding the technology platform via the development of novel, industrially viable, biodegradable polymers, innovative formulation approaches, together with studies on release profiles and durations.

Experimental Methods: The impact of polymer-based parameters, *i.e.*, content, ratio, and composition, on the API release profile, local tolerance and bio-resorption is investigated through different studies. The potential of BEPO® for small molecules is presented through the release of two APIs, *i.e.*, ivermectin and bupivacaine, analyzed by UPLC. The loco-regional tolerance after subcutaneous injection in minipigs is monitored by macroscopic skin observations and histopathology for pristine polymer depots with two injection flow rates and volumes. Finally, the bio-resorption of polymer depots *in vivo* were characterized by weight loss, GPC, NMR and DSC for 3 months and compared with *in vitro* experiments.

Results and discussion: Copolymer content and different combinations of TB and DB allow the modulation of *in vitro* and *in vivo* delivery durations between days to months. The subsequent rate of phase inversion, resulting from the DMSO release, showed to have a great impact on swelling, depot porosity, and API release. After *in vivo* injection, good local tolerance was observed in a human-relevant animal model. Finally, PEG-PLA-based depots exhibited kinetics of bio-resorption driven by polymer-based parameters, resulting from degradation by random chain scission of polyester bonds, leading to the bulk erosion of the polymer material both *in vitro* and *in vivo*. The chemical composition determined over the course of the study showed a progressive enrichment in homopolymers to the detriment of (m)PEG-PLA copolymers, with very good consistency *in vitro* and *in vivo*.

Conclusion:
The results of these studies illustrate that the API release profile and kinetics of bio-resorption of the BEPO® technology can be tuned by changing the polymeric composition with a good agreement *in vivo* and *in vitro*. BEPO® is a versatile platform offering the possibility of designing optimized LAIs with distinctive desired durations of action.

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Acknowledgments: MedinCell research members, University of Montpellier.

Hyaluronic acid aerogels prepared via freeze-thaw induced gelation

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Introduction

The biodegradability, the biocompatibility and the bioactivity of hyaluronic acid (HA) make this polymer attractive for biomedical applications such as wound dressings[1]. Biobased aerogels are reported to have a lot of potential in biomedical applications thanks to their low density, high porosity, and high specific surface area. In this work, HA hydrogels, serving as precursors for HA aerogels, were prepared using the freeze-thaw (FT) induced gelation method. The FT process allows for physical crosslinking of HA and avoids the use of toxic crosslinking agents. The influence of processing conditions on the properties and the morphology of the HA aerogels was evaluated.

Experimental Methods

HA aerogels were prepared from HA solutions using the FT induced gelation method, solvent exchange and drying with supercritical CO_2. The properties of these aerogels (e.g. specific surface area, density and porosity) were investigated as a function of HA concentration in solution, the solution pH, the number of FT cycles and the non-solvent used during solvent exchange. SEM was used to analyze the aerogel morphology.

Results and Discussion

HA aerogels (example shown in Figure 1a) were obtained with low density (< 0.2 g/cm^3), high porosity (> 90%) and high specific surface area (up to 600 m^2/g). Moreover, SEM pictures (example in Figure 1b) showed that HA aerogels contain meso- and macropores. It was demonstrated that the internal structure and the properties of HA aerogels can be tailored: for example, when the HA concentration or the number of FT cycles is increased, the HA aerogel density increases. A strong influence of solution pH was observed: at pH 2.5 (isoelectric point) the specific surface area was 500 – 600 m^2/g while at lower and higher pH it was very low.

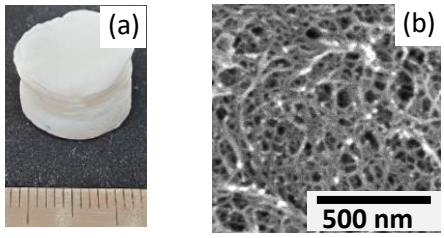

Figure 1. HA aerogel made from 1 wt% HA solution at pH 2.5, using 3 FT cycles and acetone as non-solvent: (a) photo and (b) cross-section.

Conclusions

The tunable properties of the obtained HA aerogels make them promising biomaterials, which could potentially be used for e.g. wound dressings.

Acknowledgments

The work was performed in the frame of the ANR (French National Research Agency) JCJC project '3D-AER-HYAL'. We are grateful to Pierre Ilbizian and Julien Jaxel (PERSEE, MINES Paris) for supercritical drying and to Suzanne Jacomet (CEMEF, MINES Paris) for help in SEM imaging.

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Colloidal systems of sulfur(II)-based copolymers for anti-inflammatory therapies

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Introduction

Reactive Oxygen Species (ROS), albeit necessary at homeostatic levels, lead to severe impairments when in excess [1]. ROS can be scavenged with, for example, sulfur (II)-based polymers, that can be formulated as nano-objects (high density of active groups per mass) [2].
Poly(propylene sulfide) (PPS) has been found to be quite responsive to hypochlorite, hydroxyl radicals and hydrogen peroxide [3], while poly(thioacetal) (PTA) promptly reacts also with superoxide anions [4]. Thus, their combination in a copolymer could allow a simultaneous and efficient scavenging of a wide range of the ROS spectrum, both intra and extracellularly.

Experimental Methods

Bifunctional PPS was synthesized *via* anionic ring opening polymerization [1]; PTA was obtained by a base-catalyzed (DBU or TBD) Michael-type double addition. Their copolymers (PPS_{2n}-PTA: multiblock; PPS_{2n} /PTA: statistical) were synthesized with a step-growth mechanism, starting from a purified PPS_{2n} (n=5, dispersity≈1.01) and PTA oligomers produced *in situ*. Alternating copolymers (PPS_{2n}-TA) were obtained by a one-pot PPS anion titration with an alkyne (methyl propiolate). Cell-free ROS scavenging was assessed on Nile Red-loaded nanodroplets (stabilized with Pluronic F-127).

Results and Discussion

A step-growth copolymerization between polymers (each with a typical distribution of molecular weights) implicates challenges due to reduced chain reactivity and stoichiometric control (Figure 1A). Here we show a synthesis that allows to reach $\overline{M_n}$s up to 11 kDa. The resulting copolymers were then formulated as stable nano-emulsions (Figure 1B), able to reduce levels of ROS in aqueous environment (Figure 1C). Functionalization and different end-capping of the aforementioned polymers (for cross-linked nanoparticles) is currently under investigation.

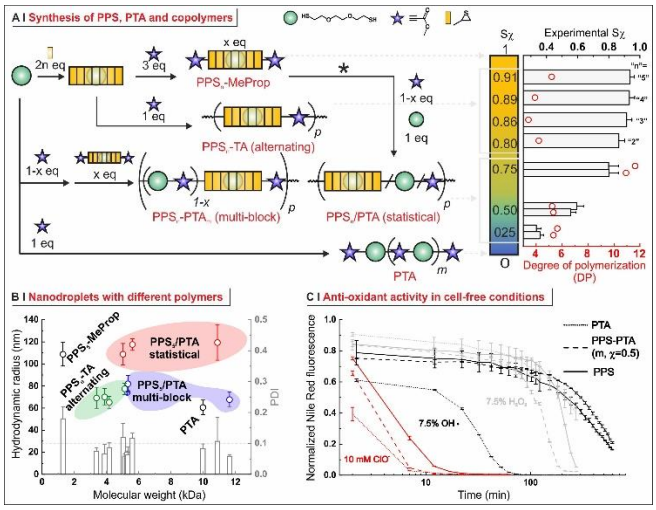

Figure 1. These synthesis require a few steps (one-pot or sequential; * = purification of the intermediate) and the DP is not extremely high (A). Even so, thanks to the high M_n, all polymers were formulated as stable nanodroplets of 60-120 nm (B; n=3) that promptly react with ROS (enhancing the polarity of the droplet environment and thus quenching Nile Red, see C; n=3).

Conclusions

These sulfur (II)-based copolymers, each with a typical ROS-scavenging specificity, are able to cover all the most widely present ROS species and their combination.

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Acknowledgments

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Dual-crosslinked degradable elastomer with self-healing properties

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Introduction

Chemically crosslinked degradable elastomers based on synthetic polyesters have been investigated for tissue engineering applications in the past years[1]. They present advantages such as a rubber-like behaviour matching with those of soft tissues and an ability to preserve 3D structure over their degradation. However, one major drawback is their inability to be healed after damage or breaking. This can be overcome by supramolecular networks whose polymer chains are linked via reversible non-covalent bonds, that act as crosslinks which bring self-healing properties to the material. In this study, our objective was to combine the mechanical and degradation properties of photo-crosslinked elastomers, with the self-healing properties of hydrogen-bonds supramolecular networks. To this aim, a degradable 8-arm star-shaped PEG-PLA block copolymer was synthesized and functionalized with methacrylic (MC) and/or catechol (CT) groups. Various degradable dual-crosslinked elastomers were then designed and the mechanical properties, self-healing efficiency (SHE), and biodegradability were evaluated.

Experimental Methods

Star-shaped copolymers were synthesized by ROP before being functionalized with methacrylate and/or catechol groups. Elastomer films were prepared by solvent evaporation and exposed to UV light for 5 minutes per side. The mechanical properties were assessed through tensile test at room temperature. To study the self-healing, the samples were cut in two pieces, that were then put together, pressed for 5 seconds and heated at 37°C for different times.

Results and Discussion

Increasing the amount of catechol improved the elongation but decreased the stress at break. Elongation and stress reached 539% and 10.7 MPa for $MC/CT_{100/0}$ respectively, against 932% and 1.8 MPa for $MC/CT_{0/100}$. $MC/CT_{75/25}$ was selected as the best compromise with intermediate values (798% and 3.4 MPa) and interesting self-healing properties. The SHE was calculated by comparing the values of elongation and stress at break of the healed samples to the original ones. SHE increased with time and $MC/CT_{75/25}$ was totally recovered after 60 minutes. SHE reached 100% of the elongation at break and 120% for the stress, which reflects the properties improvement through the dynamic reorganization of the network.

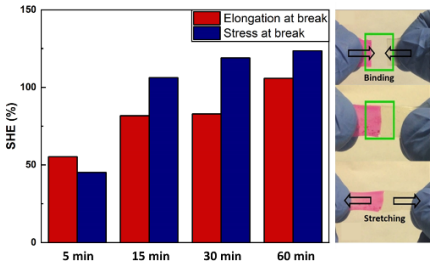

Figure 1. Evolution of SHE with time of $MC/CT_{75/25}$ and images of self-healing of a cutting film

Conclusions

In this work, we designed dual-crosslinked elastomers thanks to methacrylate and catechol functionalized star-shaped PEG-PLA polymers. The resulting elastomers exhibited mechanical properties that are compatible with soft tissues, self-healing properties at 37°C and showed to be degradable *in vitro*.

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Preparation, characterization and cytotoxicity evaluation of cellulose aerogels beads made using ionic liquid

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Introduction

Aerogels are light-weight and nanostructured porous materials with high specific surface area. Polysaccharides based aerogels are promising materials for life science applications. For example, microcrystalline cellulose (MCC) is a commonly used excipient in pharmaceutical industry, but it has to be dissolved to make shaped cellulose. The goal of this work is to develop the productions of monolith and bead-shaped cellulose aerogels using ionic liquid. The shaped aerogels physico-chemical characterizations and human cells viability in contact are reported.

Experimental Methods

MCC was dissolved in a mixture of an ionic liquid, diazabicyclo[4.3.0]non-5-enium acetate, and dimethyl sulfoxide. Cellulose solutions were shaped as monoliths and as beads by prilling, coagulated with ethanol and dried with supercritical CO_2. Specific surface area was calculated with the Brunauer-Emmett-Teller method, density by an envelope density analyzer and the inner morphology was characterized by Scanning Electron Microscopy (SEM). Human Bone Marrow Derived Mesenchymal Stem Cells (hMSC) were seeded on the aerogel surfaces and cultured in α Minimum Essential Medium for 1, 3 and 7 days. Viability of cells was determined by Hoechst coloration and live/dead assays.

Results and Discussion

We obtained cellulose aerogels monoliths and beads with similar characteristics: a specific surface area around 300 m²/g, a low density of 0.1 and a high porosity (> 90 %). Beads had a mean diameter between 0.5 and 1.5 mm and showed a monodispersed size distribution. The correlations between processing conditions of prilling (pressure, frequency, distance to the coagulation bath) and beads characteristics will be discussed. hMSCs have a high viability on the monolith aerogel surfaces. Those highly porous biomaterials could be compatible with life science applications even if potential toxicity of the ionic liquid has to be addressed.

Conclusions

We demonstrated the feasibility of making cellulose aerogels via dissolution-coagulation using ionic liquid, and their shaping on a large scale into beads via prilling process. The materials' cytocompatibility was also investigated with the aim of an environmental or biomedical application.

Acknowledgments

We are grateful to Institut Carnot MINES for the financial support of this work. We thank Pierre Ilbizian and Julien Jaxel (PERSEE, Mines Paris) for supercritical drying, Suzanne Jacomet (CEMEF, Mines Paris) for guiding in SEM imaging and Antoine Perron (GALA-RAPSODEE, IMT Mines Albi) for the prilling process.

In vitro and *in vivo* degradation of photo-crosslinked poly(trimethylene carbonate-co-ε-caprolactone) networks

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Introduction

Poly(trimethylene carbonate) (PTMC) is an amorphous, biodegradable and flexible polymer. Methacrylate-functionalized PTMC oligomers (macromers) can be photo-crosslinked into flexible and elastic networks for use as medical implants. The *in vivo* degradation of these networks is relatively slow, with a mass loss of only ca. 5% after 36 weeks for networks prepared from macromers with a molecular weight of 27 kg/mol[1]. The degradation rate can be tuned by photo-crosslinking functionalized copolymers of TMC with other monomers. Here, we present the results of degradation studies of poly(trimethylene-co-ε-caprolactone) P(TMC-co-ε-CL) networks.

Experimental Methods

Methacrylate-terminated PTMC and P(TMC-co-ε-CL) macromers were prepared by ring opening polymerization and subsequent methacrylate functionalization. TMC to ε-CL monomer ratios were: 100:0, 75:25 and 50:50. Networks were then obtained by photo-crosslinking.
In vitro degradation was assessed using a phosphate buffered solution (pH 7.4) containing Cholesterol Esterase and analysis of the samples at pre-determined intervals. The *in vivo* degradation was assessed upon subcutaneous implantation in rats.

Results and Discussion

In vitro, the degradation rates of the copolymer networks was higher than those of the homopolymer networks. The 50:50 P(TMC-co-ε-CL) network degrades at the highest rate, with a remaining mass of 33.7±5.4% after 36 weeks. Additionally, the degradation process appears to take place via surface erosion.
In vivo, the degradation rates show similar trends (Figure 1). Upon explanation at the later timepoints, 50:50 P(TMC-co-ε-CL) networks had lost their structural integrity, and remaining mass and thickness (and thus the degradation mechanism) could not be determined.

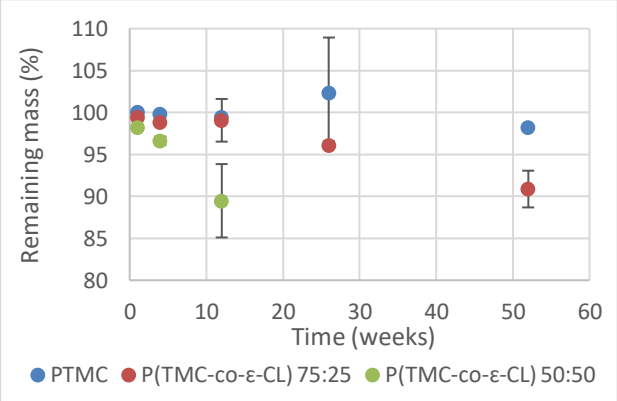

Figure 1. *In vivo* degradation of P(TMC-co-ε-CL) networks.

Conclusions

P(TMC-co-ε-CL) networks have an increased degradation rate as compared to PTMC networks, both *in vivo* and *in vitro*. *In vitro*, the networks degrade by a surface erosion process.

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Development of a 3D-printable polypeptide gel for bone regeneration

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Introduction

There is increasing interest in using 3D-printing to fabricate scaffolds that mimic bone tissue. Culturing cells on these scaffolds can induce ossification. However, challenges such as mechanical strength, neovascularization, and cell attachment remain [1]. Our group has developed a method that enables the self-assembly of elastin like polypeptides (ELP) into a supramolecular matrix capable of triggering hierarchical organised mineralization that can exhibit the mechanical properties of bone [2]. However, this method is restricted to the fabrication of membranes. The aim of this study is to establish a formulation that allows the layer-by-layer fabrication of mineralizing ELP gels for bone regeneration.

Experimental Methods

New cross-linking agents for ELP gels are explored, that provide an underlying supramolecular framework, enabling biomineralization. In the light of the aim to formulate a 3D-printable material, fast cross-linking kinetics are considered favourable. The constituents are separately dissolved in a DMF-DMSO solvent combination and subsequently mixed in a vial, using a vortex. The samples are immersed in $Ca^{2+}PO_4^{3-}$ solution (37°C, 10 days) to induce hierarchically guided mineralization. The formed mineralized structures are examined via Scanning Electron Microscopy. To provide a proof of concept, two syringes are attached to a custom 3D-printed dual extrusion system.

Results and Discussion

Immediate gelation is observed upon mixing >0.2% cross-linking agent with peptide solution. Figure 1a shows an ELP gel, extruded through a double syringe system. The macroscopic (Fig.1b, c) and nanoscopic hierarchy (Fig.1d) of the mineralized structures strongly resemble those observed in the membrane configuration [2]. By integrating custom 3D-printed components, tailored towards the gels' cross-linking kinetics, this method will be implemented into a conventional dual extrusion system.

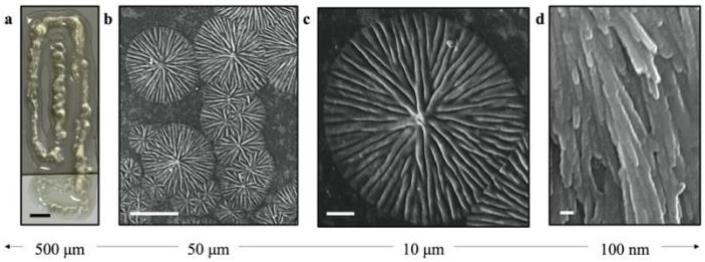

Fig.1 Hierarchically mineralized ELP gel. **a** Photograph of ELP gel before mineralization. **b** Mineralized structures grow until they meet each other. **c** Macroscopic circular structure. **d** Nanoscopic aligned nanocrystals.

Conclusions

The gelation observed in the newly designed cross-linked ELP gels and the successful biomineralization qualify the formulation for the desired application.

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Acknowledgments

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Graphene/polyacrylonitrile membranes induce spontaneous breast cancer cell spheroid formation

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Introduction

Despite the great interest arising from the tumor spheroids capacity to recapitulate characteristic features of in vivo tumors and their use as models, their experimental protocols are time consuming and costly, therefore their routine use is not widespread[1].

This work aims at evaluating if the immobilization of high concentrations of graphene nanoplatelets in polyacrylonitrile (PAN) substrates allows the modulation of the migratory response of cancer cells[2] and spontaneous recapitulation of breast cancer cell spheroids using a simple cell culture protocol.

Experimental Methods

PAN-graphene flat membranes were prepared by phase inversion from a polymer solution containing 10 wt% of PAN, 10 wt% of graphene (Av-PLAT-7, Avanzare, Spain) in N-methyl pyrrolidone (NMP). PAN/G10 membranes were characterized by scanning electron microscopy (SEM), Raman spectroscopy, goniometry and electrical impedance. MCF7 breast cancer cells were cultured, and migratory and differentiation responses were analyzed using epithelial cell markers and wound healing assays, and gene and protein expression by Real-time quantitative PCR and Western blotting, respectively.

Results and Discussion

Raman spectroscopy showed high presence of agglomerates in the PAN/G10 membranes (Figures 1A). Breast cancer MCF7 cells were localized on top of graphene rich regions and presented activation of the E-cadherin expression, reorganization of cells into solid cellular islets with adherens junctions (Figure 1B), and the inhibition of their migratory capacity.

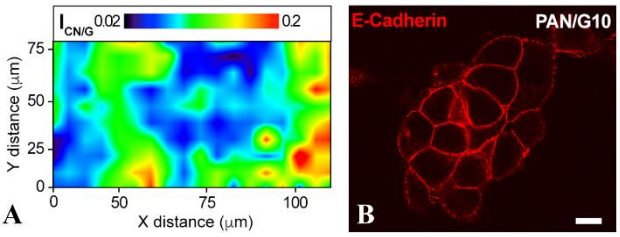

Figure 1. A) Raman intensity ratio of nitrile (CN) characteristic band of PAN and G band of graphene ($I_{CN/G}$), and B) Confocal microscopy images showing E-cadherin expression in cell membrane of MCF7 breast cancer cells, and cellular aggregation induced by PAN/G10 (72 h). Scale bar: 15 μm.

Conclusions

This study highlights the potential of the 2D scaffolds PAN/G10 as migrastatic agents that may provide new strategies for cancer therapy.

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Acknowledgments

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Poly(1,5-dioxepan-2-one) (PDXO) networks prepared by photocrosslinking

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Introduction

Poly(1,5-dioxepan-2-one) (PDXO) is an amorphous polymer with a low glass transition temperature. Compared to other biodegradable polyesters, it is relatively hydrophilic and therefore of great interest in a variety of biomedical applications. The mechanical properties of PDXO are rather disappointing, but can be improved by (block) copolymerization and network formation [1-4]. Also photo-crosslinked networks have been prepared from acrylate-functionalized PDXO precursors [5], but their mechanical characteristics were not evaluated. Here we describe the synthesis and properties of photo-crosslinked PDXO networks.

Experimental Methods

DXO was synthesized by Baeyer-Villiger oxidation of tetrahydro-4*H*-pyran-4-one. DXO was purified by recrystallization from ether, and polymerized in a N_2 atmosphere at 110 ^oC for 24 hrs using 1,6-hexanediol as initiator and SnOct2 as catalyst. Functionalization was done by reaction with 2-isocyanato-ethyl methacrylate. Networks were obtained by photo cross-linking PDXO macromer films at 365 nm for 30 min, using Irgacure 2959 as initiator.

All polymers and macromers were analyzed by ^{1}H-NMR. The networks were characterized by their swelling behaviour in dichloromethane, their uptake of water and their thermal- and mechanical properties.

Results and Discussion

The degree of functionalization of the PDXO macromers decreased with increasing molar mass.

Table 1. Characteristics of PDXO polymers, macromers and obtained photocrosslinked networks.

	Mn (kg/mol)	DOF (%)	Tg (^oC)	Gel (%)	Q
PDXO-1	0.7	100	n.d.	91.3	1.8
PDXO-10	9.7	100	-41.1	93.3	3.5
PDXO-30	30.4	68	-42.0	70.1	10.9
PDXO-43	43.3	72	-42.8	54.0	16.3

Table 1 also shows that Tg of the amorphous networks slightly increased with decreasing crosslink density.

Table 2 shows that values of the elasticity modulus, tensile strength and elongation at break of the PDXO networks are much influenced by the molar mass of the macromer used. Water uptake increases with macromer molar mass and has a large effect on mechanical properties.

Table 2. Characteristics of obtained PDXO networks

	Water uptake (%)	E (MPa)	σ (MPa)	ε (%)
PDXO-1	dry	74.8	7.2	22.8
PDXO-10	dry	5.6	2.1	63.5
PDXO-30	dry	0.3	0.5	276.4
PDXO-43	dry	0.1	0.2	211.2
PDXO-1	18.2	45.9	3.4	10.6
PDXO-10	26.7	4.3	2.9	102.6
PDXO-30	33.0	0.2	0.4	284.8
PDXO-43	41.2	0.1	0.2	201.9

Conclusions

PDXO networks with appropriate mechanical characteristics can readily be prepared by photo crosslinking methacrylate-functionalized PDXO precursors. The precursor molar mass has a large effect on the resulting physical- and mechanical properties.

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Development of novel hybrid hydrogels based on chitosan/poly(2-oxazoline)s-block-poly(2-oxazine)s for bio-based aerogels fabrication

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Introduction

Bio-based aerogels are dry 3D networks and highly porous materials, which are discussed for applications in wound dressing (Groult et al., 2021). 3D-printing is a novel 3D fabrication method, supposed to offer precise positioning of materials and hydrogels, and can be employed to fabricate bio-aerogels with improved 3D architecture. Natural polymers (e.g. chitosan) possess favorable biological properties, but poor rheological performance and poor printability. On the other hand, synthetic polymers present favorable rheological properties and sub-optimal biocompatibility. Thus, developing hybrid hydrogels using synthetic polymers as rheology modifiers for natural polymers can potentially overcome the limitations (Hu et al., 2022).

Chitosan (CHI) is widely used in biomedical applications due to its favorable biological properties such as biocompatibility and antimicrobial activities (Sahranavard et al., 2020). To address its poor printability, a synthetic block copolymer comprising poly(2-methyl-2-oxazoline) and thermoresponsive poly(2-n-propyl-2-oxazine) (nPrOzi) (POx-b-POzi) can be used as a sacrificial rheology modifier and porogen. POx-b-POzi is a thermoresponsive and cytocompatible polymer and its proper rheological properties such as strong shear thinning are of great interest for 3D-printing (Lorson et al., 2017).

Experimental Methods

A thorough rheological characterization supplements the printability studies on hybrid hydrogel comprising POx-b-POzi and CHI. After printing and crosslinking CHI with NaOH, the synthetic hydrogel is dissolved and ^{1}H-NMR measurements examine the residual POx-b-POzi. Subsequently, aerogels are prepared via solvent exchange to ethanol, following by sc CO_2 drying. Furthermore, aerogel bulk density and specific surface area were determined, and FESEM was performed to study the morphology of aerogels (Groult et al., 2021).

Results and Discussion

A highly porous bio-aerogel is fabricated via 3D-printing of a novel hybrid hydrogel to be used for wound dressing application. The synthetic component drastically improves printability and allows crosslinking of CHI with exceptional shape fidelity.

Conclusions

We report a hybrid approach containing POx-b-POzi and CHI that shows a feasibility to be successfully employed for 3D-printing to fabricate 3D network bio-based aerogels.

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3D Printing of Porous PCL-*b*-PTMC-*b*-PCL and Nano-Apatite Composite Structures

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Introduction

Several calcium phosphates have bone-forming capabilities [1,2]. In this respect composites with poly(trimethylene carbonate) (PTMC) are of great interest, as the polymer shows surface erosion *in vivo* without forming acidic degradation products. Previously, we have used stereolithography to prepare designed composite PTMC and nano-apatite (nAp) scaffolds for bone tissue engineering [3].
Stereolithography is not as widely available to researchers as other additive manufacturing techniques. Therefore, we developed new thermo-plastic elastomeric composites based on PCL-*b*-PTMC-*b*-PCL triblock copolymers and nAp, and prepared porous structures using extrusion-based additive manufacturing.

Experimental Methods

A PCL-*b*-PTMC-*b*-PCL triblock copolymer was synthesized by sequentially polymerizing TMC and ε-CL. NMR showed that Mn of the PTMC and PCL blocks were resp. 38 kg/mol and 19.5 kg/mol. Composites with homogenously distributed nAp were prepared by dispersing nAp particles (5-25µm sized agglomerates of needle-like hydroxyapatite crystals of 200-400 nm long and 20-50 nm wide) in chloroform. The polymer was then dissolved in the dispersion, precipitated in methanol and dried.
To prepare porous composite films and composite inks for 3D printing, the precipitate was dissolved in ethylene carbonate at 50 ^{o}C at a total solids content of 25 wt%. Ethylene carbonate melts at 35 ^{o}C and is soluble in water, allowing the preparation of porous films and 3D printed structures after cooling and extraction with water. Designed structures were made using a BioBot 1 extrusion-based 3D printer.

Results and Discussion

Flexible, porous films containing 0, 20 and 40 wt% nAp were prepared. Their porosity ranged from 71 to 74%, with pores approximately 14 µm wide and 25 µm long. With an increase in nAp content, the E modulus increased from 0.06 to 0.26 MPa and the elongation at break decreased from 34 to 19 %.
3D printing resulted in porous composite structures with a (designed) macroporosity having pores of 530-620 µm, and micropores of 5-30 µm that resulted from the crystallization of ethylene carbonate. SEM images also showed the presence of nanopores with sizes of 200 to 500 nm.

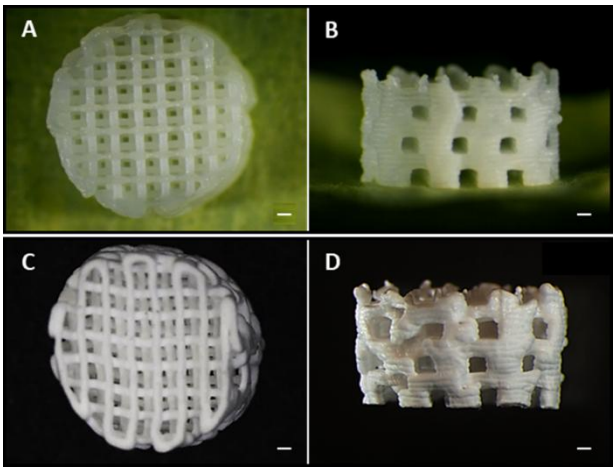

Figure 1. Light microscopy images of porous composite structures of PCL-b-PTMC-b-PCL and 20 wt% nAp. 3D printing was done from solutions in crystallizable ethylene carbonate. The images were obtained before extracting the crystallized solvent (A, B) and after extracting the solvent with water and drying (C, D). Scale bar is 300 µm.

Conclusions

This study demonstrates the feasibility of preparing porous flexible composite structures from PTMC-based block copolymers and nano-apatite via extrusion-based 3D-printing. The potential bone forming ability will be evaluated in future studies.

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Additive Manufacturing of Patient-Specific Mandibular Bioimplants

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Introduction

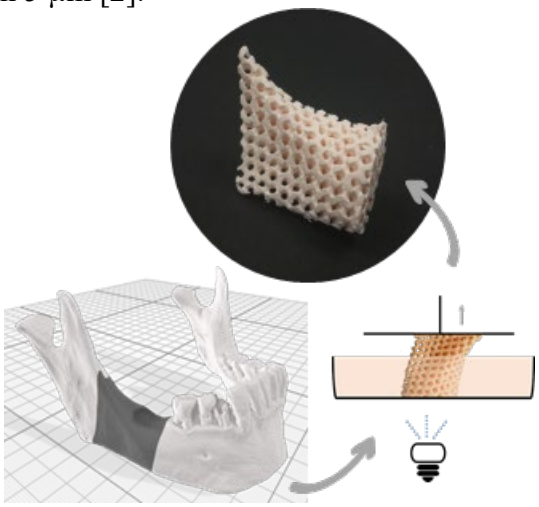
Composite implants combining bioresorbable polymers and osteoconductive calcium phosphate ceramics present a synthetic option to replace and repair bone tissue. Particularly extensive craniomaxillofacial bone reconstruction cases benefit from patients-specific implants (PSI) that can predictably and accurately restore both the anatomical form of the patient and the physiological function of the tissue. In this poster we summarize our recent years work [1,2] on developing bioactive composite implants (bioimplants) for large mandibular defects including a proof-of concept study in minipigs.

Experimental Methods

Scaffolds of cross-linked poly(trimethylene carbonate) (PTMC) networks and tricalcium phosphate (TCP) were manufactured and characterized to determine material structure-property correlation [1] and biocompatibility [2]. PSI bioimplants were finally tested in vivo in a large mandibular continuity defect model in Göttingen minipigs. [2]

Results and Discussion

Previous studies have established that PTMC+TCP implants show biocompatibility and promote osteogenesis. The minipig study further confirmed that bioimplants establish good osteoconduction and signs of osteoinductivity. However, the bioimplants were also associated with higher incidence of infection. Hypothetically, large amounts of the ceramic could have resulted in local microenvironment changes in the implant such as sub-physiologic pH and calcium and phosphate concentration gradients or in macrophage phagocytosis of particles smaller than 5 µm [2].



Conclusions

The product development work including implementation of full production workflow of large implants and in vivo minipig study highlights the importance of testing implants in clinically relevant models and at the in situ reconstruction site to truly test the clinical performance.

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The Covalent Entrapment of Fragile Compounds into Biodegradable Polymeric Micelles via a Native Chemical Ligation Crosslinker

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Introduction

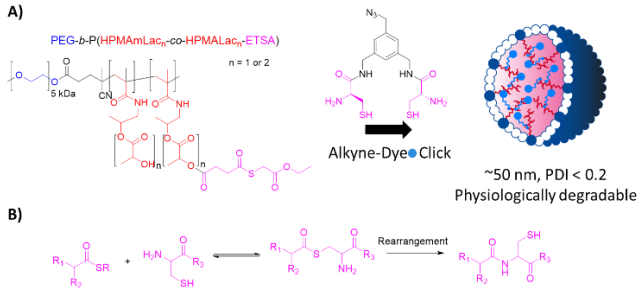
HPMA-lactate based thermosensitive nanoparticles were developed as a biodegradable core-crosslinked micelle platform for applications in oncology with a docetaxel formulation having undergone phase II clinical trials. Due to the free radical chemistry being employed during the synthesis of these core-crosslinked micelles, the current system is limited to only a number of active compounds that can be covalently entrapped. Most new generation pharmaceuticals are peptide or protein based and they can be damaged by free radical chemistry. Several fluorescent dyes for potential imaging purposes are also incompatible with the current platform. We therefore developed an alternative crosslinking method for HPMA-lactate based polymers that employs native chemical ligation (NCL, a bio-orthogonal reaction between *N*-terminal cysteine residues and thioesters).

Experimental Methods

A novel trifunctional crosslinker with two cysteine handles required for NCL and an azide moiety for the click loading of fluorescent dyes or prodrugs was synthesized. We synthesized and modified the thermosensitive PEG-*b*-P(HPMAmLac$_1$-*co*-HPMAmLac$_2$) polymers with complementary thioester handles for NCL. Core crosslinked polymeric micelles (CCPMs) were obtained by crosslinking at 37^{o}C in 100 mM phosphate buffer. CCPM stability was investigated by DLS and UHPLC. Fluorescent dyes were entrapped by use of click chemistry and cytocompatibility and uptake of the CPCMs were studied using PC3 cells.

Results and Discussion

CCPMs of varying sizes were obtained from the polymer library and the 50 nm lead formulation was selected. The CCPMs were stable to surfactant challenge and could be hydrolyzed under physiological conditions. Dye loaded CCPMs were found to have high uptake in PC3 cells in line with a previous HPMA-lactate based platform[1], no cytotoxicity up to a tested concentration of 2 mg/mL polymer content was detected.



Scheme 1. Structures and synthesis of CCPMs with entrapped dyes using a novel trifunctional crosslinker, B) NCL mechanism.

Conclusions

These fluorescent dye entrapped CCPMs can be applied in diagnostic imaging and the chemistry developed in this work serves as a steppingstone towards covalently entrapped fragile drug compounds with tunable release from CCPMs

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Antiviral activity of quaternized chitosan derivatives: structure-activity relationships

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Introduction

Modified cationic polysaccharides have a huge potential in several applications, and they exhibit substantial beneficial properties. This research investigates how structural differences affect the antimicrobial activity; glycidyl trimethyl-ammonium chloride (GTMAC) has proven antimicrobial activity and we introduced this quaternary compound to chitosan to investigate its less known antiviral activity. In this research, we compared the structural properties of GTMAC to [2-(acryloyloxy)ethyl]-trimethyl-ammonium chloride (AETMAC), a novel compound in this field, to compare the structural differences of quaternary chitosan and its antiviral activity relationship.

Experimental

In this research, we prepared a series of quaternized chitosan and carboxymethyl chitosan (CMC) compounds with both GTMAC and AETMAC, either homogeneously (single or double quaternized) or heterogeneously quaternized and evaluated their structural properties and antiviral activity relationship[1].

Results and discussion

The viral activity is dependent on the structural properties of chitosan, such as, introduced quaternary ammonium compound, chain length, degree of quaternization, hydrophobicity, functional groups introduced and solubility. It was found that, in addition to charge density of quaternary chitosan, other mechanisms affect the antiviral activity of the compounds as well. It is proposed that the higher hydrophobicity and longer alkyl chains of AETMAC improves the antiviral activity further. However, the anti-viral behaviour depends on the type of virus, time of exposure and environmental conditions; here, our compounds showed higher antiviral activity against the enveloped virus φ6 compared to non-enveloped φX174, see Figure 1.

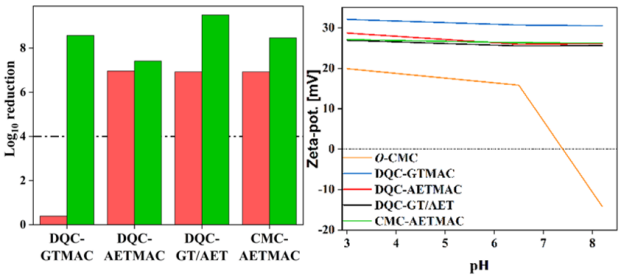

Figure 1. *Left:* φX174 (red) and φ6 (green) bacteriophages viral reduction of quaternized chitosan samples in non-dried conditions, where a log reduction of 4 or greater is considered antiviral. *Right*: Zeta potential of CMC and quaternized chitosan, where the free amine of CMC is deprotonated at neutral and alkaline pH, hence the charge changes from positive to negative, whereas quaternized compounds have a permanent positive charge. A positive charge is essential since most viruses are found to carry a negative net charge.

Conclusion

Chitosan quaternized with AETMAC improved the viral reduction significantly, compared to chitosan quaternized with GTMAC. These findings are of great interest in several fields, such as medical applications, personal protective equipment or in the water treatment industry.

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Sterilization of bio-based aerogels by supercritical CO_2 technology

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Introduction

Aerogels are lightweight materials with low density, high specific surface area and highly open porosity which can be produced from many sources with customized morphologies. Namely, aerogels made of natural polymers are promising candidates for a wide range of biomedical applications, including their use as synthetic bone grafts. Any implantable medical device must be sterile to avoid the outcome of potentially life-threatening infections. Sterilization of bio-based aerogels with supercritical carbon dioxide ($scCO_2$) is herein presented as an effective technique operating at mild working conditions that leads to solvent-free materials [1].

Experimental Methods

Starch and alginate aerogels were manufactured following reported protocols [1]. The $scCO_2$ sterilization process was performed using spore strips of *Bacillus pumilus* as biological indicator to evaluate the sterility. Bio-based aerogels were characterized before and after the sterilization process by SEM, N_2 adsorption-desorption test and gas pycnometry to evaluate any relevant change in the nanostructure.

Results and Discussion

The sterilization conditions used in this work (140 bar, 39ºC, 150 min) achieved 6-logarithmic reductions of the initial spore population, and the aerogel nanostructure was preserved after the process (Fig. 1). The mild conditions herein used could avoid the physicochemical degradation of aerogels observed in previous studies [1].

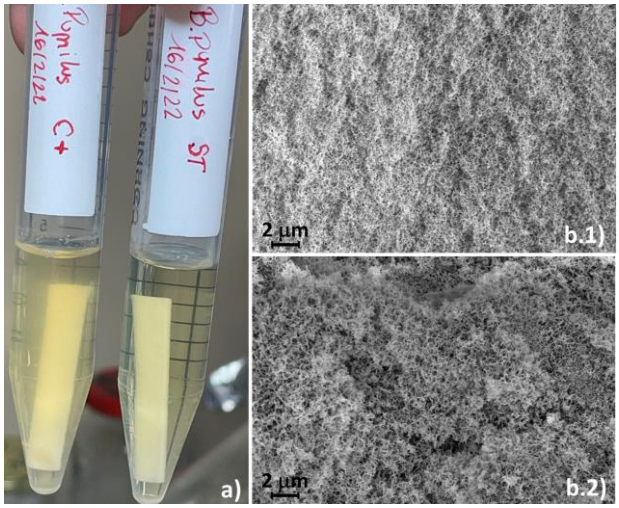

Figure 1. a) TSA tubes with *B. pumilus* strips: positive control (C+) and sterilized (ST); Sterilized b.1) alginate and b.2) starch aerogels

Conclusions

A $scCO_2$ sterilization method compatible with the treatment of bio-based aerogels was successfully developed. Aerogels from different sources with varying morphologies were manufactured and sterilized. Sterilized materials maintained their morphologies and therefore its future biomedical performance.

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Design of hybrid polymer/peptide nanofibers for soft tissues regeneration

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Introduction

Nanofibers are excellent biomimetic supports for tissue regeneration since they mimic the architecture of the natural extracellular matrix. However, PLA is an inert polymer and therefore not active on cells. This work aims to create PLA nanofibers that, in addition to this guiding role, could modulate cellular behavior. For this purpose, we have created the possibility to covalently combine functionalized PLA with bioactive peptides directly during the electrospinning process in order to create a bioactive 3D network constituting the nanofibers.

Experimental Methods

We synthesized 4-arms star-PLAs by ring opening polymerization of DL-lactid on pentaerythritol. StarPLAs were functionalized by reacting IPTES on polymers. The polymers were characterized by SEC, H-NMR and DOSY. Peptide 1 (IKVAV) was synthetized by standard Fmoc SPPS on Chlorotrityl-resin. After functionalization by IPTES under basic and anhydrous conditions, the obtained peptide was characterized by LC/MS. PLAs were dissolved in TFE at concentrations ranging 5 to 20 wt % with HCL to activate the sol-gel process.

Results and Discussion

First, the molecular weights of the 4-arms star-PLAs were close to the theoretical values and their dispersity were low. Moreover, the functionalization step add 4 PTES functions to the 4-arm star-PLAs and 2 functions to the peptide (100% functionalization rate). Then, we produced hybrid nanofibers by reacting silylated peptides with silylated PLAs via sol-gel process during electrospinning and fine-tuned this process in order to increase the crosslinking of the network. In order to facilitate electrospinning, we added a linear-PLA diluent. However, as the amount of diluent increased, the cross-linking of the nanofibers was lower. Interestingly, the crosslinking of the nanofibers was improved with the use of low molecular weight polymers due to a high content of crosslinking groups. Indeed, gel fractions were increased from 40 to 61% by replacing the Star-PLA25k-PTES by Star-PLA12k-PTES or Star-PLA5k-PTES. We then introduced the peptide 1 inside the electrospun solution to add biological properties to the nanofibers. The addition of 0.1% and 1% of bifunctionalized peptide 1 increased the crosslinking density and in consequence, the nanofibers stiffness and strength. Finally, the biological properties of hybrid nanofibers on skin fibroblasts were studied and confirmed that these materials are non-cytotoxic and enhance L929 cells proliferation.

Conclusions

In conclusion, we developed a new process to obtain nanofibers composed of a hybrid three-dimensional network that contain degradable polymers covalently bonded with bioactive peptides. The polymers and peptides can be modified, like legos, to fit different target tissues.

Acknowledgments

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3D printing of textile-like biodegradable membranes: effects of printing parameters on mechanical and structural properties

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Introduction

A wide range of clinical issues can be addressed by biomedical textiles. While external uses include wound dressings or bandages for instance, implantable textiles can serve for general surgery, orthopedic, cardiovascular and plastic surgeries, or tissue engineering [1-2]. Yet, textile manufacturing is complex and alternative process to produce textile-like membranes would be beneficial to explore new designs and material compositions. Recently, under-extrusion fused deposition modeling (FDM) printing has been reported for the production of quasi-textiles [3]. However, impact of the manufacturing conditions on the membrane features was not fully explored. In this study, influence of these parameters on the membrane mechanical and structural properties is evaluated.

Experimental Methods

Membranes of poly(lactic acid) were printed by FDM (Stream 20Pro MK2, Volumic 3D) and imaged by optical microscopy (Zeiss Axio Vert.A1). Their mechanical properties were investigated in tensile mode (INSTRON 3343, steady speed of 5 mm/min, 170x22 mm specimens, n=2).

Results and Discussion

Textile-like membranes were produced by under-extrusion regime in FDM, a technique were the extrusion multiplier (EM), a parameter related to the volume of melt polymer extruded per time unit, is optimized to obtain a non-continuous filament deposition [3]. Varying the printing speed and extrusion multiplier values was found to impact on the membrane tensile properties (Figure 1.A,B). When these parameters were set to their optimal values (best mechanical properties), a membrane with regular porosity was obtained (Figure 1.C). The porous architecture of the membrane was also influenced by the printing speed, as lowering the later induced an axial elongation of the open pores. On a mechanical point of view, the membrane obtained at 40 mm/s with an extrusion multiplier of 0.8 showed a Young Modulus 2.5 higher compared to a commercial PET woven mesh that is used in surgical and medical device applications.

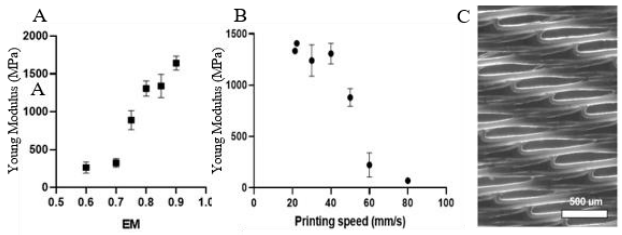

Figure 1. Apparent Young modulus of the membranes obtained at a constant A) printing speed (40 mm/s) and B) extrusion multiplier (EM=0.8). C) Optical microscopy of the membrane obtained at 40 mm/s, EM=0.8

Conclusions

Controlling the under-extrusion FDM printing of PLA enables the production of thin (<0.3 mm) and flexible membranes with potential applications as biomedical textiles. This easy manufacturing process is also versatile, and will therefore be applied to different biocompatible and biodegradable materials in future studies.

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Acknowledgments

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Decellularized mammary gland bioinks for the development of 3D breast cancer models

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Introduction

The tumor microenvironment (TME) plays a crucial role in tumor progression, and current *in vitro* models cannot mimic its complexity. 3D printing is a promising technology for developing *in vitro* models that recreate the TME. The extracellular matrix (ECM) role in tumor progression[1] has motivated the fabrication of biomimetic bioinks using decellularized tissues-derived matrices (TDMs). However, their rheological properties prevent their bioprinting. This work **aims** to develop a breast TDM-like bioink suitable for bioprinting breast cancer models.

Experimental Methods

Porcine mammary glands were decellularized and its composition evaluated. TDM was digested with pepsin, printed with a bioplotter (RegenHU) and crosslinked. The addition of rheological modifiers and collagen 1 (Col1), overexpressed in breast cancer, was also evaluated. Bioinks' printability and mechanical properties were determined. For bioprinting models, breast cancer cells (BCCs) were dispersed in the bioink. The cellular survival, proliferation, morphology, e-cadherine expression, and doxorubicin efficacy were studied. For statistical analysis, one-way or two-way ANOVAs were run.

Results and Discussion

Porcine mammary glands were decellularized and the resulting TDM was rich in glycosaminoglycans and collagen. The addition of rheological modifiers allowed the TDM bioprinting with suitable printability and optimal stiffness to recreate the tumor. BCCs proliferated in bioprinted hydrogels forming spheroids with a low e-cadherin expression. The addition of Col1 improved the bioink printability, increased cellular proliferation and reduced the resistance to doxorubicin. TDM bioinks also allowed BCCs and stromal cells bioprinting. Further research will demonstrate the suitability of TDM bioinks for the bioprinting of models containing stromal and cancer cells.

Conclusions

Breast TDM bioinks closely recreate the tumor ECM stiffness and composition and allow the bioprinting of breast cancer models. Bioinks can be further tuned with Col1 increasing the BCCs proliferation and doxorubicin sensitivity.

References

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