AFPM

Advanced Functional Polymers for Medicine 2019

June 05th - 07th, 2019
Aalto University
Finland

Abstract Book

Advanced Functional Polymers for Medicine

2019

Welcome to AFPM 2019 at Aalto University, Finland. In your hand you are holding the abstract book for the 2019 APFM conference. The purpose of the series of AFPM conferences 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. We hope you will enjoy the excellent set of scientific speakers and leading experts presenting this year. We would like to thank all invited speakers and poster presenters for taking the time to visit Finland and AFPM 2019. Thank you also to all of our sponsors who make the conference possible.

If you have any questions or concerns, do not hesitate to contact the local organizing commite. You can find them portrayed below.

Welcome to Finland!

Chairs AFPM 2019 :

Prof. Dr. Jukka Seppälä, Aalto University, Finland
Prof. Dr. Andreas Lendlein, Helmholtz-Zentrum Geesthacht, Teltow, Germany
Prof. Dr. Dirk W. Grijpma, University of Twente, The Netherlands

Local organising committee AFPM 2019:

Jukka Seppälä

Bas van Bochove

Kasper Dienel

Arja Tuohino-Chance

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Advanced Functional Polymers For Medicine 2019

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9:00-9:30	Dr. Andre Poot University of Twente, Netherlands	*Preparation of micro-porous poly(trimethylene carbonate) structures by stereolithography and particle leaching*	
9:30-10:00	Prof. Anna Finne Wistrand KTH Royal Institute of Technology, Sweden	*Designing Fiber based Degradable 3D porous Scaffolds suitable for Soft Tissue Engineering*	
10:00-10:30	Prof. Ashok Kumar IIT Kanpur, India	*Bioengineering polymers for orthopedic and nerve regeneration applications*	
10:30-11:00	**Coffee break**		
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16:30-17:00	Prof. Ana Paula Pêgo University of Porto, Portugal	*Engineering the Future of Targeted Therapies to the Nervous System with nanoBiomaterials*	
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9:30-10:00	Prof. Pekka Vallittu University of Turku, Finland	*Fiber-Reinforced Composites for Implant Applications*
10:00-10:30	Dr. Shady Farah Massachusetts Institute of Technology, USA	*Engineering Bioactive Functional Polymers for Long-Term Therapies*
10:30-11:00	Coffee break	
11:00-11:30	Dr. Julien Gautrot Queen Mary University London, UK	*The Visco-Elasticity of 2D Protein Networks – Applications for Stem Cell Expansion*
11:30-12:00	Prof. Marjo Yli-Perttula University of Helsinki, Finland	*Clinical investigation of the suitability of wood based nanofibrillar cellulose for skin graft donor site treatment*
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Afternoon program for those who have enrolled for the Finnish experience

14:00-16:00	Extended program Finnish Forest Chemistry
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20:00-…..	Experience Helsinki City Centre

Lecture Abstracts

Preparation of micro-porous poly(trimethylene carbonate) structures by stereolithography and particle leaching

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Introduction

Additive manufacturing (AM) allows for the preparation of designed tissue engineering scaffolds with optimal properties concerning porosity, pore interconnectivity, pore size and pore geometry. Of all AM techniques, stereolithography (SLA) is the most versatile and accurate method allowing structures to be built at a resolution of 15-300 μm. Although for many applications pores sizes in this range or somewhat larger are suitable, micro-porosity in the scaffold structures is advantageous in view of prolonged nutritional supply throughout the scaffold after implantation. Examples are scaffolds for bone and cartilage regeneration. Likewise, the walls of an artificial capillary network need to be micro-porous for the delivery of nutrients to cells. In the present study, we aimed to introduce micro-porosity in poly(trimethylene carbonate) (PTMC) structures by combining SLA and particle leaching.

Experimental Methods

Three-armed PTMC oligomers with a M_n of 4 kg/mol were synthesized by ring-opening polymerization of TMC. The PTMC oligomers were reacted with methacrylic anhydride to yield PTMC macromers with methacrylate end-groups (PTMC-MA). $CaCO_3$ with an average particle size of 0.56 μm was used as porogen. A dispersion of calculated amounts of $CaCO_3$ and PTMC-MA in chloroform was homogenized and precipitated in cold ethanol to yield a composite that was dried to constant weight. Resins were prepared by homogenizing the composite in propylene carbonate and adding TPO-L photo-initiator and Orasol Orange G dye. PTMC structures containing 45 vol% $CaCO_3$ were printed using an Ember Autodesk digital light processing stereolithograph at a pixel resolution of 50x50 μm and a step height of 50 μm. Built structures were extracted in chloroform/acetone (1:1 v/v) solutions, immersed in chloroform containing Irgacure 2959, dried and post-cured in a UV cabinet. The $CaCO_3$ particles were leached in watery solutions containing 3.7 vol% HCl which was finally replaced by water.

Results and Discussion

PTMC structures were successfully designed and built, as shown in Figure 1 for a tubular structure. The printed tubes had an inner diameter of 500 μm and a wall thickness of 280 μm. After extraction, post-curing and porogen leaching, porous structures were obtained as shown in Figure 2. Pore sizes as determined by SEM amounted to 0.64 ± 0.45 μm for the inside of the tubes, 0.46 ± 0.28 μm for the outside and 0.63 ± 0.58 μm for the cross-section. As the leached structures shrunk upon drying, pore sizes in the wet state are probably larger. The porosity in the wet state as determined by gravimetry was 58 ± 2 %.

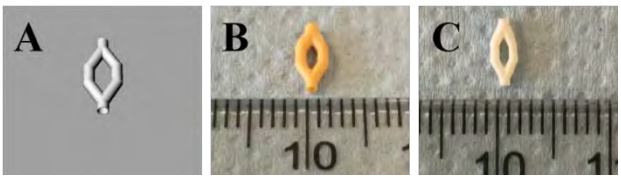

Figure 1. A: designed tubular structure, B: built structure before extraction, C: built structure after extraction, post-curing and porogen leaching.

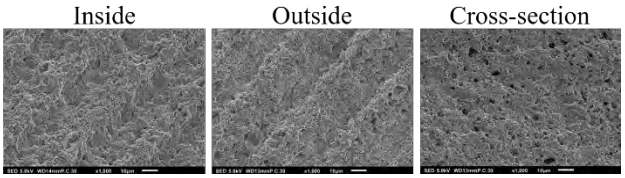

Figure 2. SEM images of tubular wall after extraction, post-curing and porogen leaching. Scale bar is 10 μm.

Conclusion

Designed micro-porous PTMC structures were successfully prepared by SLA and particle leaching using $CaCO_3$ as porogen.

Acknowledgments

The authors thank Huizhou Foryou Medical Devices Company for kindly providing TMC monomer and the Chinese Scholarship Council for financial support.

Designing Fiber based Degradable 3D porous Scaffolds suitable for Soft Tissue Engineering

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Introduction

Successful results in tissue engineering are dependent upon the interaction between the medical device and the biological environment. We have designed scaffolds with certain mechanical stability and degradability, size, surface roughness, functionality and porosity to provide a microenvironment for sufficient cell–cell interaction, cell migration, proliferation and differentiation.

We have most recently focused on the production process, used every step to generate a device with beneficial material properties. Knitted devices are used in many clinical applications today and 3D printed devices are getting closer to the clinical environment. These processes have in common that they are fiber based and that the device is fabricated with precision and repeatability.

We have assessed the rheological properties and melt stability of medical grade degradable polymers, produced fibers in order to understand the structure-processing relationship. [1-3] We have defined the important material changes that take place during the process from polymer melt to fiber formation and following post treatment. We have varied the structure-processing relationship and the architecture of the fiber based device and correlated this with mechanical properties and degradation profile. This knowledge have subsequently been used to 1) design fibers that resorb fast when the mechanical properties are lost 2) print soft and pliable 3D scaffolds.

The results give important understanding in how to produce successful fiber based degradable medical device for soft tissue engineering.

A holistic approach is critical for covering the contribution of material issues, structural design issues and processing issues and the interactions among them.

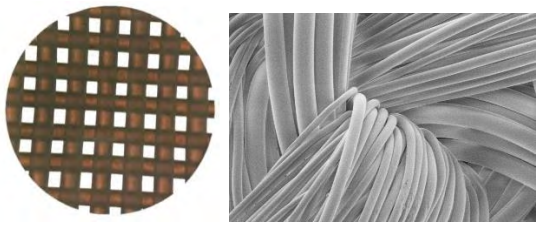

Figure 1. Fiber based degradable scaffolds

References

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Acknowledgments

Financial support from Swedish Foundation of Strategic Research (RMA 15-0010) is acknowledged.

Bioengineering polymers for orthopedic and nerve regeneration applications

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Introduction

With the growing advancement in the field of biotechnology, biomaterial science, tissue engineering and regenerative medicine, and their tremendous application in biomedical science, bioengineering of the developed tissue engineered (TE) implants is one of the benchmarks for their translation. We have been developing biomimetic TE scaffolds which have shown promising application and translational potential for bone and nerve regeneration.

Experimental Methods

We use cryogels, which are supermacroporous polymeric hydrogels synthesised under frozen conditions with a defined architecture to mimic different tissues structure such as bone, nerve, liver and cardiac. We develop composites of different polymers using cryogelation, 3D printing and electrospinning to regenerate these tissue. In-vitro analysis is carried out to understand their biocompatibility as well as functionality in terms of growth and differentiation. To further demonstrate their regenerative potential, we carryout in-vivo analysis of these developed scaffolds in rat and rabbit models.

Results and Discussion

For musculoskeletal regeneration, cryogels were employed for repair of surgically created critical size cranial bone defect as well as subchondral defect in knee joint in rabbits. The cryogels showed promising results at macroscopic, microscopic and gene levels. To functionalize these materials for bone repair and regeneration, nanohydroxyapatite based scaffolds were developed along with calcium sulphate hemihydrate. In-vitro as well as in-vivo evaluation of these materials showed enhanced bone formation at the site of defect. These materials also performed as an efficient delivery vehicle to deliver bioactive molecules (bisphosphonates and bone morphogenetic proteins) at the defect site and to enhance their osteopromotive properties. We have also developed 3D printed PTMC-hydroxyapatite scaffolds for bone regeneration. For nerve regeneration we have developed biomimetic 3D printed and electrospun nerve guidance channels (NGCs) with aligned cryogel matrix. These NGCs have shown promising results in regeneration of a critical size nerve defect. We were able to obtain not only the morphological aspects of the damaged nerve but could establish the electrophysiological functionality. Recently we have explored the use of stem cell derived exosomes for repair and regeneration of bone and nerve tissues.

Conclusions

Development of functionalized biomimetic scaffolds will pave way to develop patient specific regenerative and TE scaffolds for repair and regeneration of the damaged tissues.

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Acknowledgments

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Light-Guided Micropatterning of Polymers for Organ Models

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Introduction

Light-guided chemical patterning of polymers is a versatile tool for producing complex 3D cell culture environments, both through stereolithographic 3D printing of bulk shape and in local post- modification of printed objects to control cell behavior such as selective adhesion. We explore the fundamental limits in achievable 3D spatial detail in complex polymer topologies at the sub-100 µm length scale required to mimic organ microenvironments and engineer perfusable synthetic microvasculature. In particular, we address the possibilities and limitations of combining multiple patterned polymer materials in stereolithographically 3D printed objects by use of orthogonal polymerization mechanisms (free radical and cationic, respectively) during printing to offer freedom in design of local mechanical and diffusion properties of organ models.

Experimental Methods

A home-built stereolithographic printing system offers exposure with a lateral resolution of 10.8 µm at 365 nm and 460 nm.[1] We use distinct light patterns at the two wavelengths to activate either cationic (at 365 nm) or free radical (at 460 nm) photoinitiators in a solution of epoxy and poly(ethylene glycol)-diacrylate (PEG-diacrylate) monomers.[2] Both crosslinked materials have been tested to be cell compatible after repeated washing. The 3D printed objects are analyzed structurally by optical (fluorescence) microscopy and chemically by IR and Raman spectroscopy.

Results and Discussion

Optical microscopy of printed 3D checkerboard test structures shows that spatial detail below 100 µm is possible (Figure 1, upper panel), although with some loss of detail. Our previous work showed that illumination of bulk reagent solution at 365 nm results in activation of both cationic and free radical polymerization.[2] Chemical analysis of individual exposed layers shows spatially selective enrichment of the free-radical based PEG-diacrylate away from the illumination source, which compromises mechanical stability of the neighboring 3D printed layers, in addition to significant continued "dark polymerization" of the epoxy component. Optimization of the reagent solution and the exposure timing may significantly reduce such inhomogeneities to enable even higher spatial detail.

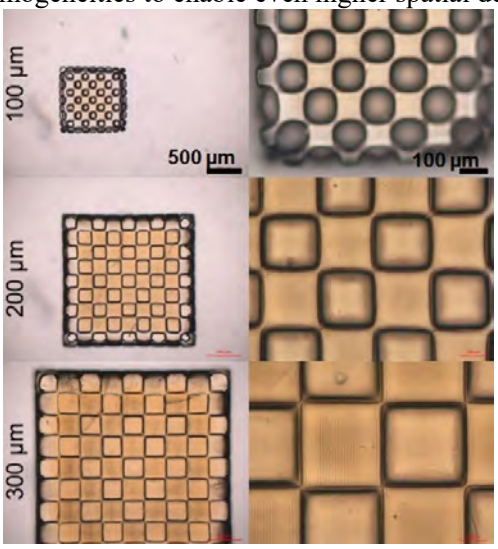

Figure 1. Evaluation of the spatial resolution of dual-material printing: Optical micrographs of 3D checkerboard patterns with alternating epoxy and PEG-diacrylate cubes of design sizes $(100\ \mu m)^3$, $(200\ \mu m)^3$, and $(300\ \mu m)^3$.

Conclusions

Dual-material 3D printing with sub-100 µm spatial resolution of importance for organ model engineering is possible. Optimization of the reagent solution composition and the exposure timing will likely offer further reduction in feature sizes.

References

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Acknowledgments

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Additive Manufactured Polymer-based Biomaterials

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[1]Institute of Applied Synthetic Chemistry, TU Wien, Vienna, Austria.

Introduction

Additive manufacturing technologies, esp. based on lithography (L-AMTs), open the possibility to provide patient-specific implants with feature resolutions down to the sub-micron range.[1,2] Vinyl esters (VE, incl. vinyl carbonates) have been established as biocompatible precursor for photopolymers,[3] which are the basis for L-AMTs. Esp. in combination with thiols (thiol-ene chemistry) VEs attain reactivities towards photopolymerization comparable with those of acrylates, the benchmark materials for L-AMTs, however, VEs have an about two orders of magnitudes lower toxicity.[4] Moreover, VEs have very favorable degradation products. Low molecular weight polyvinyl alcohol and carbon acids can be excreted easily and residual monomers hydrolyze to acetic aldehyde.[5]

Owing their characteristic polymer architecture, photopolymers have a very low toughness in general. However, by application of toughness enhancers, high molecular weight co-monomers as well as chain transfer agents, toughness can be increased to values enabling the fixation of the implants by screws.[6]

Experimental Methods

Synthesis of VEs is either accomplished by lipase (CAL-B) catalyzed transesterification reactions of desired building blocks (e.g., polycaprolactone telechelics, PCL)[4] with divinyl adipate or by condensation reaction with vinyl chloroformate.[6]

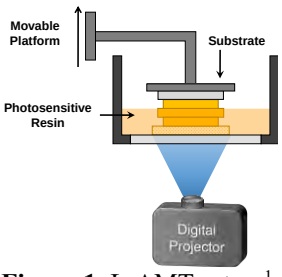

Figure 1. L-AMT setup.[1]

Besides classical mechanical characterization of cured specimens, RT-NIR-photorheology was employed to investigate the photopolymerization characteristics of formulations with different monomers and additives.[7]

For additive manufacturing a digital light processing (DLP) L-AMT system with upside-down set-up and an InVision® WUXGA 1080p light engine with 460 nm LED was used (Figure 1).[8]

Results and Discussion

The facile synthesis VEs enables the generation of a toolbox system (Figure 2) containing a variety of components for the formulation of materials with different properties.

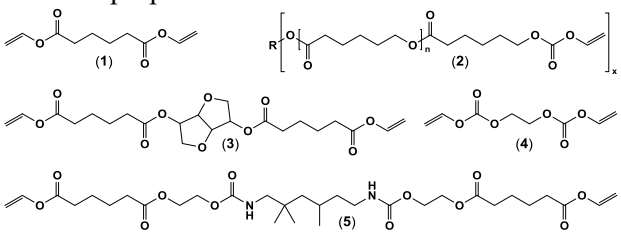

Figure 2. Vinyl ester (VE) based monomers: divinyl adipate (**1**), x-armed PCL-VE (**2**), gycitol based VE (**3**), ethylene glycol based VE (**4**), urethane based VE (**5**).[3-6]

An appropriate combination of base monomers (VEs and thiols), additives to adjust the toughness and degradation characteristics, and e.g. hydroxyapatite for bone regeneration, enable the manufacturing of patient specific implants by L-AMT.

References

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Acknowledgments

The financial support by the KLS Martin Group is gratefully acknowledged.

Poly(trimethylene carbonate) / TCP scaffolds; 3D Printing, Bioactivity and Degradation Control

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Additive manufacturing of bioactive polymers and composites opens up new avenues for precisely designed and customized biomaterials. Stereolithography is based on the principle of photocuring of prepolymers in the pre-designed voxel-areas. Here, stereolithography was applied to produce scaffolds for tissue regeneration. Individually designed TCP filled scaffolds based on photocuring poly(trimethylene carbonate) (PTMC) was produced. Filler contents up to 40% particle form TCP could be successfully used in stereolithography. Following initial printing tests, upscaling was achieved by prototyping and resin optimization. Utilizing CT data, patient specific bone regeneration implants were printed for a large bone defects. Properties of thus created biomaterials will be presented.

Results on materials properties, rheology and preliminary results on *in vivo* experiments will be presented.

PTMC degrades via enzymatic surface erosion. The degradation of photocured PTMC is slow with maximum mass loss of 3.7% *in vivo* after 36 weeks. Increase in the degradation rate can be achieved by using copolymers with ε-caprolactone. Additionally, an increase in degradation rate may be achieved by the preparation of PTMC-anhydride networks.

Preliminary results of *in vitro* degradation studies with such networks will be presented.

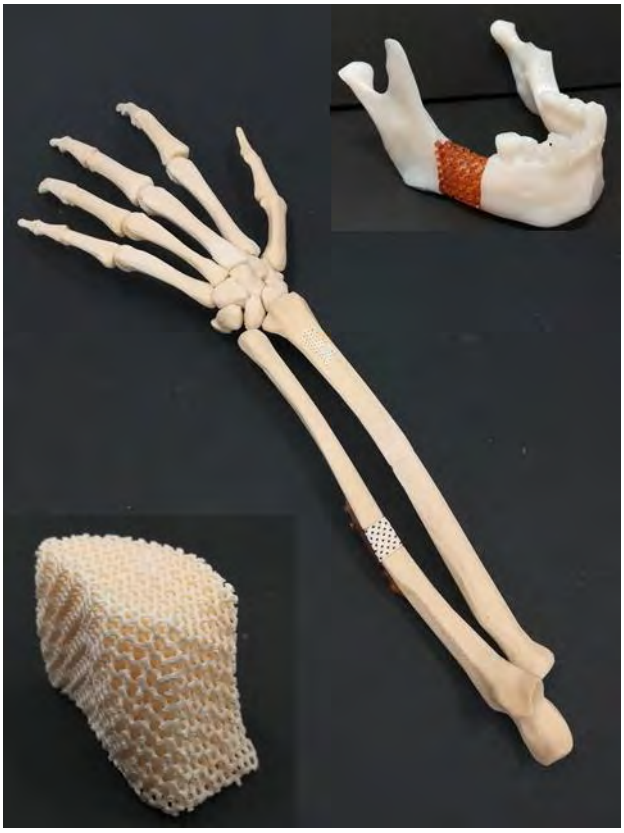

Figure 1. Prototypes of PTMC/TCP composite bone regeneration scaffolds.

Acknowledgments
This work made use of Aalto University Bioeconomy Facilities.

Nucleic Acid Delivery by Thermosensitive Polyplexes

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Introduction

Nowadays, complexation of nucleic acids with polymeric carriers into polyplexes is a well-known approach to enable their delivery and uptake by the target cells. Various polymer designs and corresponding polymerization techniques can be used for the synthesis of tailor-made macromolecules for nucleic acid delivery systems. In this study, we combined cationic and thermosensitive properties into the polymer design and investigated the corresponding polyplex properties upon complexation with pDNA and siRNA, respectively.

Experimental Methods

NPD triblock copolymers consisting of a thermosensitive *N*-isopropylacrylamide (PNIPAM, N), a hydrophilic poly(ethylene glycol) (PEG, P) and a cationic 2-(dimethylamino)ethyl methacrylate (PDMAEMA, D) block with different block lengths were prepared using a hetero-functional PEG macroinitiator [1]. Polyplexes were prepared using the NPD polymers complexed with pDNA and siRNA. Physical properties including size, zeta-potential and stability were evaluated at different temperatures. Cell viability and transfection efficiencies of NPD polyplexes were compared with non-thermosensitive PD polyplexes.

Results and Discussion

We found that there is a critical balance between the electrostatic and hydrophobic interactions between the multifunctional polymer and pDNA at temperatures above the CP. If the length of the cationic block and the N/P ratio are high enough, the electrostatic interactions between pDNA and the cationic block of the polymer are superior over the hydrophobic thermosensitive interactions, resulting in stable polyplex nanostructures. Furthermore, the NPD polyplexes show a lower ζ-potential than the PD-based polyplexes, which is most likely due to the additional shielding of the PNIPAM blocks. Moreover, the presence of thermosensitive blocks in NPD-based polyplexes resulted in better cytocompatibility compared to PD-based polyplexes with similar efficiencies of delivering cargo into HeLa cells.[2]

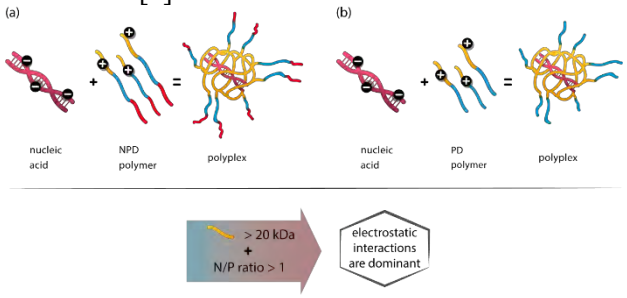

Figure 1. Polyplex structures using the NPD (a) or PD (b) polymer. If the length of the cationic block (D) is above 20 kDa and the N/P charge ratio higher than 1, the electrostatic interactions between the pDNA and the D-block of the polymer are dominating over the hydrophobic thermosensitive interactions.

Conclusions

These results provide new insights into the design of polymers for advanced drug delivery systems, such as polyplex-releasing thermosensitive hydrogel systems for the controlled and local delivery of nucleic acids.

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Engineering the Future of Targeted Therapies to the Nervous System with nanoBiomaterials

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Nervous system problems are common and encompass a large spectrum of traumatic injuries, diseases or iatrogenic lesions. The poor regenerative capacity, particularly in the case of the central nervous system (CNS), cannot be attributed to an intrinsic inability of neurons to sprout and re-grow after injury, as axons are able to regenerate in the presence of a permissive growth environment. One of the challenges facing the neuroscience field is the development of effective therapies that can enhance the regenerative capacity of the nervous system based on the advances achieved in basic research.

We have been dedicated to using nano-enabled solutions to the design of new therapeutic approaches based on biomaterials to promote neuroprotection and neuroregeneration both in the context of the peripheral and central nervous system lesions.

In this talk, two main strategies will be presented that embody two of the main lines of research of my group:

i) the design of biomaterial-based nanocarriers for targeted nucleic acid delivery to neurons, for which we have been exploring both chitosan[1] and a new family of fully biodegradable dendrimers proposed by us[2] as vectors.
ii) the design of cell instructive matrices for neural stem cell delivery in the spinal cord[3] based on PEG hydrogels functionalized with human laminin tethered in a site-specific manner.

Emphasis will also be given to the application of bioimaging tools to assess the potential of the developed systems[3-6].

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Polysulfides as anti-oxidant and anti-inflammatory agents

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Introduction

Biologically relevant oxidants (Reactive Oxygen Species, ROS) express a variety of roles, ranging from survival factors and activators, promoters of mobility to inflammatory agents and directly cytotoxic molecules. Indeed, most inflammatory pathologies are characterized by a strongly oxidizing environment caused by high concentrations of (enzymatically produced) ROS, and this feature can be used to design materials that by virtue of their sensitive to oxidation can have an inflammation-responsive performance[1].

Our group has pioneered the field of ROS-responsive polymers by developing organic polysulfides (poly(1,2-alkylene sulfide)s); these polymers can be fashioned as nanomaterials (nanoparticles, micelles, also polymer vesicles), where the conversion of thioethers into sulfoxides or sulfones induces strong physico-chemical changes (e.g. swelling or dissolution), e.g. accelerating the release of encapsulated drugs[2]. In recent years we have focused on the links between polysulfide macromolecular architecture (primary structure, branching) and oxidative response kinetics[3,4] and formation of higher order structures[5].

Results and Discussion

In this communication we specifically discuss the biochemical and biomedical aspects of the ROS-scavenging activity of polysulfides.

In particular, our recent *in vitro* and *in vivo* data demonstrate the direct anti-inflammatory behaviour of polysulfides, which appears to be predominantly based on extracellular ROS scavenging.

For example, using cross-linked and PEGylated nanoparticles as the main material (Figure 1), we have shown very significant amelioration of the consequences of cerebral stroke[6]. Even if these ROS-scavenging nanoparticles were administered 3h after stroke, at a point when most damages caused by injury after reperfusion have already occurred, yet they significantly sped up the recovery and reduced inflammatory cerebral complications.

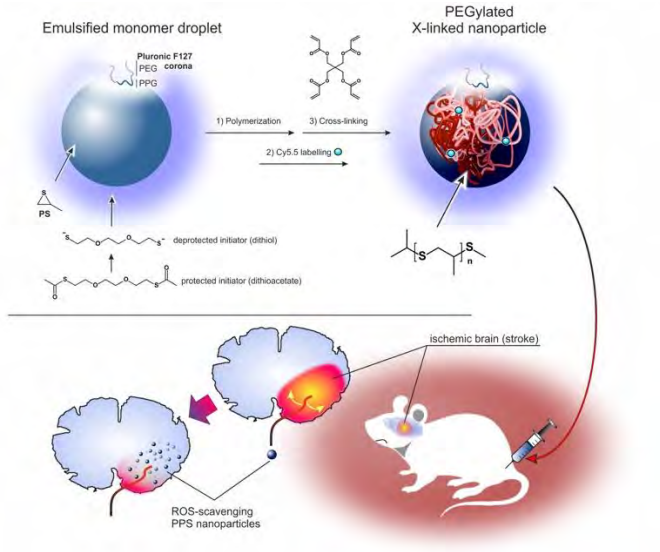

Figure 1. Top: polysulfide nanoparticle preparation (emulsion polymerization followed by labelling and cross-linking). Bottom: sketch of the administration of the nanoparticles 3h after producing stroke in murine models.

Conclusions

The take-home message of the communication is the strong anti-inflammatory performance of polysulfide nanoparticles, as a consequence of extracellular ROS scavenging.

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Biomaterials for the Early Detection and Treatment of Disease

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Introduction

Biomaterials can be used for the early detection and treatment of disease. For early detection, imaging contrast agents functionalized with specific targeting moieties can be used as '*in vivo* histology'. This approach is particularly useful in the detection and prevention of surgical adhesions, which are bands of tissue arising from abdominal surgeries. Complications of surgical adhesions include small bowel obstruction and infertility, and the annual cost of adhesion-related complications to the US healthcare system is estimated to be as high as $5 billion. Ultrasound contrast agents were first developed as blood contrast agents and were not designed to last for long periods of time. For targeting applications, it is important for ultrasound contrast agents to have long-term stability. We have recently developed a novel formulation of ultrasound contrast agents using polydiacetylene-based polymerizable lipids that are stable for up to four days[1], and the targeted contrast agents bind to components found during the initial stages of adhesion formation[2]. These studies have been validated in an ischemic button model of surgical adhesions in Sprague-Dawley rats.

Biomaterials can also be used for the treatment of disease. There remains a major challenge in developing surgical solutions for children with congenital heart disease. Current solutions involve multiple staged surgeries because the implants do not grow with the child. We have developed several methods using thermos-responsive polymers such as poly-Nisopropylacrylamide (PNIPAAm) to generate cell sheets or enzymatic degradation of polymers to generate layered tissue patches that mimic the cellular organization of native vessels[3]. We have further developed a bioMEMS device that can be used to assess physiological function of these tissue-engineered constructs with patient data input.

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Non-isocyanate polyurethanes (NIPU): a green strategy towards biomaterials

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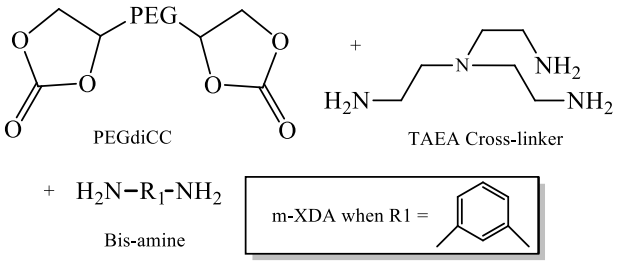
Introduction

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 poly(hydroxyurethanes) (PHU) by polyaddition of diamines with CO_2-sourced bis(cyclic carbonate)s has emerged as one of the most promising.

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 polyurethane hydrogels and composites as they are of special interests and use for biomedical drug eluting implants.

Scheme 1. Starting formulations for PHU



Experimental Methods

The PEGdiCC was obtained by carbonatation of the corresponding epoxide with CO_2.[1] The PEGdiCC (1.5 g, 2.44 mmol, 1 eq.), bis-amine (mXDA (0.225 mL, 1.71 mmol, 0.7 eq.) and crosslinker (TAEA (0.073 mL, 0.49 mmol, 0.2 eq.) formulations were mixed for 10 min then cured for 24 h at 60°C.

Montmorillonite (3 or 5 wt% of Cloisite 30B) were dispersed in the formulation for PHU composites.

Results and Discussion

After cross-linking, the PHU hydrogels were produced by immersion in water and characterized by compression tests. Material with the optimum properties were obtained from PHU hydrogels with a water content of 83% and strain at break around 60% by using 0.2 equivalent of TAEA.

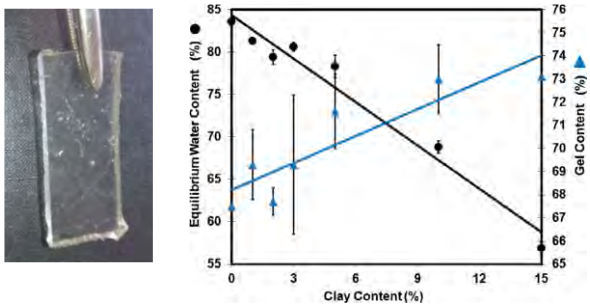


Figure 1. Picture of PHU hydrogel (left) and equilibrium water content and gel content of different nanocomposite PHU hydrogels (right).

Conclusions

By using an isocyanate-free process, PHU hydrogels have been obtained with improved mechanical properties when clay-composites are used. These materials are possible candidates for applications such as wound dressing, drug eluting implants and scaffolds for tissue engineering even if biocompatibility studies have still to be performed.

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Degradable Star Block Copolymers as Highly Versatile Architectures for Biomedical Applications

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Introduction

Star macromolecular architectures, although known for many years, have recently gained increasing visibility in the frame of biomedical applications.[1] In this contribution, we will present some recent research of our group focusing on the use of degradable star block copolymers for various medical applications. Highlight is given to the versatility of such architectures that offer opportunities for functionalization to generate advanced biomaterials.

Experimental Methods

Star block copolymers containing a PEG core and PCL or PLA arms with various compositions, number of arms and molecular weights were prepared by ring opening polymerization starting from star PEG macroinitiators. The resulting star copolymers were functionalized either on their chains ends using classical coupling strategies, or on the polyester backbone using a 2 steps activation/substitution approach.

Results and Discussion

The first system of interest consists of 8 arm PEG)$_8$-PCL star block copolymer micelles that are stabilized via π-π stacking. Benzyl groups were introduced on the PCL arms via thiol-yne photoaddition of benzyl merpactan to yield PEG)$_8$-PCL(B). In comparison with unmodified PEG)$_8$-PCL micelles, PEG)$_8$-PCL(B) micelles exhibited a 15-fold lower CMC, a 15-fold smaller size and a 50 % higher drug loading and encapsulation efficiency. Whereas the PEG)$_8$-PCL micelles showed significant aggregation during in vitro cytotoxicity experiments, the PEG)$_8$-PCL(B) micelles showed no signs of aggregation and were capable of solubilizing high concentrations of curcumin, resulting in a significant cytotoxicity towards MCF-7.[2] In a second approach, more hydrophobic PEG)$_8$-PCL were synthesized to yield more homogeneous and transparent hydrogels compared to their linear triblock counterparts. The introduction of an amide or an ester group between the PEG core and the PCL arms further allowed to modulate the hydrogels storage modulus and in vitro stability with an excellent resistance against hydrolytic degradation.[3] In a final approach, we developed a universal macromolecular photo-crosslinker consisting in a star-shaped multi(aryl-azide) PEG)$_8$-PLA(AA) copolymers that has the ability to efficiently crosslink any polymer containing C-H bonds independently of its molecular weight and without the need for pre-functionalization.[4] High crosslinking efficiencies of PLA-Puronic® matrix were obtained with PEG)$_8$-PLA(AA) compared to low molecular bis- or octa(aryl-azide) photo-crosslinkers (below 15%). Optimal conditions were used to yield crosslinked electrospun microfibers (1-2 µm) resulting in biocompatible and highly elastomeric scaffolds (ε_y>100%) compared to uncrosslinked scaffolds (ε_y<10%). The degradation rate of the scaffolds was controlled over time depending on the blend content, which offers new opportunities to prepare degradable elastomeric scaffolds for soft-tissue reconstruction.

Conclusions

Degradable star block copolymers offer a large variety of properties and chemical modification opportunities of interest for drug delivery, soft-matter and tissue engineering applications.

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Designing Materials with Multiple Functions with regard to structure-function as well as function-function relations

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The successful usage of polymeric materials strongly depends on their ability to fulfill the specific and often complex demands of applications. Multifunctional materials that allow tailoring of properties and functions including the ability to respond to internal/external stimuli or to controlling elements can substantially contribute to application-driven material solutions. Multifunctionality is obtained by integrating several functions in one material, especially those that seem to be contradictionary. In a system approach the fundamental understanding of function-structure relationships and the ability to control function-function relations build the competence for realizing required modern applications in healthcare, robotics, aerospace, or smart textiles.

Cell-instruction by materials refers to their ability to modulate behavioral and functional changes in contacting cells in a predictable manner. Physical principles can be derived from extracellular matrix (ECM) and ECM-cell interaction and provide inspiration for designing geometries and selecting suitable mechanics of materials [1,2]. The starting point are typically specific functions, which are created in an information based design approach. Polymeric materials intended for temporary use are ideally transient-by-design, wherefore degradability of polymers as a material function is targeted. Exploring thin polymer films spread at the water-air interface by applying Langmuir monolayer degradation techniques proved to be a valuable approach that is fast and requires material only in the milligram scale [3,4].

Porous shape-memory polymers offer large recovery strains upon compaction, where the pore-size distribution controls shape-memory properties on the macro- and microscale of polymeric foams. [5].

The ´one time shape change` limitation of classical shape-memory polymers was overcome with the realization of shape-memory polymer actuators, which can repetitively change their shape under stress-free conditions. Such soft actuators also possess the unique feature of re-programmability related to shape changing geometry and switching temperature. The thermally controlled reversible actuation can occur many times [6].

Orthogonal multifunctionality is realized when several functions can be nearly independently controlled. Orthogonal functions were achieved in a device combining shape-memory effect, diffusion-controlled drug release and degradability. An example for orthogonal function are soft actuators equipped with a self-healing capability [7].

Sequential multifunctionality relies on a series of functions, where the effect (output) of one function is the input of the next function. Sequentially coupled soft actuators enable a non-contact control by alternating magnetic fields [8,9]. Container tubes equipped with a shape-switching capacity for diameter reduction allow on-demand release by NIR light [10].

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Fabrication and characterization of soft-molecular imprinted electrospun scaffolds for tissue regeneration applications

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Introduction

The fabrication of bioactive scaffolds able to mimic the in vivo cellular microenvironment is a challenge for regenerative medicine [1]. The creation of sites for the selective binding of specific endogenous proteins represents an attractive strategy to fabricate scaffolds able to elicit specific cell response [2]. Here, electrospinning (ESP) and soft-molecular imprinting (soft-MI) techniques were combined to fabricate a soft-molecular imprinted electrospun bioactive scaffold (SMIES) for tissue regeneration. The fabricated SMIESs were characterized in term of morphology, roughness, wettability and rebound ability for the selected molecules.

Experimental Methods

Figure 1 summarizes the fabrication process of a SMIES. Firstly, a polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning, 10:1 (w/w)) mold was fabricated as described in [3] using a master patterned with lines (100 µm length, at a distance of 50 µm). The mold was then functionalized as described in [3] using a FITC-albumin and TRITC-lectin solution (1 µg/ml in deionized water). The functionalized PDMS mold was used as a target for the ESP process using a 4% w/v PLGA (Boehringer-Ingelheim) solution in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (Sigma Aldrich, The Netherlands) at a flow rate of 1 ml/h, voltage of 20 kV and needle-collector distance of 20 cm for 4h. The SMIES's rebinding ability was analyzed by fluorescence microscope (non-inverted NIKON E600) after dipping it FITC- albumin or TRITC-lectin water solution (1 µg/ml) and a triple washing in water. A Philips XL ESEM-FEG was used to observe the morphology of the SMIESs. Roughness was evaluated via atomic force microscopy. Samples' wettability was evaluated via contact angle.

Results and Discussion

SMIESs showed uniform fibers with diameters of 571±70 nm, and a porosity of 94.2±2.4%. Rebounding, roughness and wettability results are shown in figure 1 b), c), d), e), f), and h).

Conclusions

We presented the possibility to imprint nanofibers to create bioactive ESP scaffolds for regenerative medicine applications. Further studies are in progress to go deepen in the characterization of the fabricated SMIESs and to imprint grow factors in order to promote cellular differentiation.

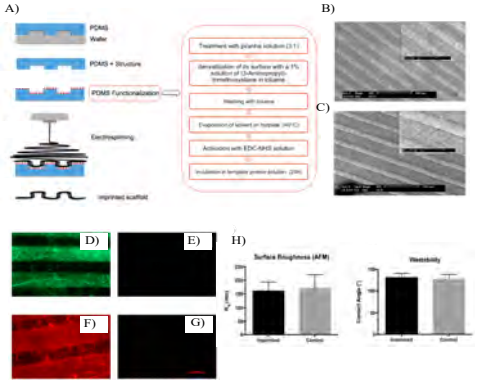

Figure 1. SMIES's fabrication process (a); SEM images of the functionalized mold and of SMIES (b,c); FITC-albumin or TRITC-lectin imprinted SMIESs and controls (d,e,f,g); surface roughness and wettability were similar for SMIESs and non-imprinted PLGA scaffold (h).

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Soft electronic and robotic systems from resilient yet biocompatible and degradable materials

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Nature inspired a broad spectrum of bio-mimetic systems – from soft actuators to perceptive electronic skins – capable of sensing and adapting to their complex erratic environments. Yet, they are missing a feature of nature's designs: biodegradability. Soft electronic and robotic devices that degrade at the end of their life cycle reduce electronic waste and are paramount for a sustainable future. At the same time, medical and bioelectronics technologies have to address hygiene requirements. We introduce materials and methods including tough yet biodegradable biogels for soft systems that facilitate a broad range of applications, from transient wearable electronics to metabolizable soft robots. These embodiments are reversibly stretchable, are able to heal and are resistant to dehydration. Our forms of soft electronics and robots – built from resilient biogels with tunable mechanical properties – are designed for prolonged operation in ambient conditions without fatigue, but fully degrade after use through biological triggers. Electronic skins merged with imperceptible foil technologies provide sensory feedback such as pressure, strain, temperature and humidity sensing in combination with untethered data processing and communication through a recyclable on-board computation unit. Such advances in the synthesis of biodegradable, mechanically tough and stable iono-and hydrogels may bring bionic soft systems a step closer to nature.

Composite Gelatin-Alginate Hydrogels as Multifunctional Bioadhesive Materials

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Introduction

In the past two decades tissue adhesives and sealants have revolutionized hemostasis and wound management in traumatic and surgical injuries. Various synthetic adhesives and biological-driven formulations are clinically utilized either as an adjunct to conventional wound closure techniques, such as suturing, or as a replacement to them. They are increasingly gaining more popularity in diverse areas of clinical applications, such as medical sealants, drug delivery systems, scaffolds for tissue engineering and dental and bone applications.

Bioadhesives is a research topic of very high challenge in terms of materials science because they must be able to be bind tissues in hemorrhagic environment and also to facilitate healing and maintain biocompatibility. Degradable bioadhesives such as cyanoacrylate glues, protein-based bioadhesives, polyethylene glycol sealants and fibrin tissue adhesives, have been developed and used. However, none of the suggested solutions provide all desired properties. Novel tissue adhesives based on the natural polymers gelatin and alginate, and loaded with functional materials were developed and studied by us. They act as composite hydrogels and will unique characteristics

Experimental Methods

The bonding strength was studied in both, tensile and lap shear modes, as well as the sealing ability of the formulations. Their viscosity was measured using a controlled stress rheometer. The gelation time and swelling degree were measured, as well as the biocompatibility of the formulations, which was evaluated on human dermal fibroblast cell according to the ISO 10993 standard.

Results and Discussion

The combination of gelatin, alginate and a carbodiimide crosslinking agent resulted in high bonding strength and sealing ability, high biocompatibility and controllable gelation time and viscosity, and therefore can be used for various specific applications. Unique bioadhesives were achieved when loaded the basic formulations with functional additives, such as hemostatic agents, drug molecules and reinforcing fibers (Fig. 1).

When loaded the bioadhesives with hemostatic agents, nano-structuring resulted in superior properties compared to micro-structuring. Incorporation of reinforcing fibers in the bioadhesives resulted in improvement of the cohesive strength of the composite hydrogel and therefore increased the sealing ability.

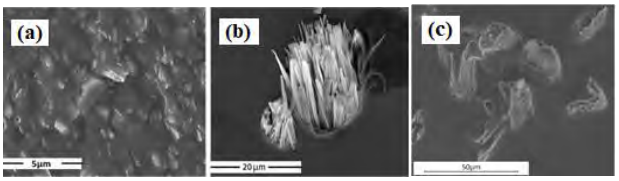

Figure 1. Environmental SEM micrographs showing the structure of the composite bioadhesives loaded with: (a) The hemostatic agent kaolin [1], (b) the analgesic drug bupivacaine, (c) reinforcing cellulose fibers [2].

Conclusions

Bioadhesives based on natural polymers which also include functional fillers are novel. They actually behave as composite bioadhesives, and thus present a new concept of adhesive biomaterials. The understanding of the relationships between formulation parameters, structure and the resulting relevant in-vitro properties and in-vivo functioning, are of great scientific and medical relevance. They are expected to provide new solutions to basic needs in various medical fields.

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Hydrogels and Hybrid Materials for Cardiac and Neural 3D *In Vitro* Models

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Introduction

Tissue engineering (TE) has now been studied some decades and the aim is to create living tissue for *in vivo* using a combination of biomaterial scaffolds, live tissue-specific cells, and biochemical factors [1]. This has been proven to be a multicomponent and complex task and thus in the recent years there has grown relevant interest to develop *in vitro* tissue and organoids for disease modeling, toxicology, and for the study of developmental biology to reveal complexicity of TE.

To study human central nervous system and cardiac system development, function and dysfunction as well as diseases, *in vitro* models could offer new insights and knowledge. To meet the goal of functional models, neurons and cardiomyocytes of human origin should be cultured in a reproducible and reliable manner. Importantly, to better mimic *in vivo*-like growth and maturation of human neuronal and cardiac cells *in vitro*, three-dimensional (3D) cultures with appropriate scaffolds are needed instead of 2D cultured models [2].

For *in vitro* models, the crucial aspects of the material selection are that material offers a natural environment for the cells and it allows interaction between and to cells. In this concept, hydrogels (HG) are also considered as scaffolds since they may mimic well original extracellular matrix (ECM). Not only the mechanotransduction but also other physical as well as chemical and biochemical factors are important for the correct choice of scaffold material. For our models, several different HGs we synthesized and studied in 2D the *in vitro* cytocompatibility of them with neural and cardiac hPSC-derived cells. They were applied in 3D with the cells and created hybrid materials to create relevant *in vitro* tissue models.

Experimental Methods

Experimental methods for HGs included synthesis of them and analysis of the structure and rheological and mechanical properties. After thorough characterization without cells, they were studied in cell culture conditions in 2D and in 3D for cytotoxicity (live/dead staining), viability and functionality using specific methods for both neuronal (NC) and cardiac (CC) cells. For cardiac cell 3D culture, polyethylene trephtalate (PET) fabrics were used for aligned topography.

Results and Discussion

Several gellan gum (GG) and hyaluronan (HA) based hydrogels and blends with proteins were synthesized/prepared and characterized. GG was purified, bioamine crosslinkers were compared, and GG was functionalized for cell attachment. GG with gelatin was found suitable for cardiac cells. Also, topography of PET-fabrics improved the quality of the CCs.

NCs favored HA-based HGs instead of GG-HGs but laminin was found to improve in both cases. This study also gave an interpenetrated network (IPN) HG, which is supporting and enabling adhesion of the cells by its collagen I component. We were able to synthesize the IPN-HG by developing and utilizing a Principal Component Analysis (PCA) tool to analyze measured and indexed data comparing several human-based NC types and multiple hydrogel compositions.

Conclusions

3D cell culture environment is better mimicking tissue than 2D but it requires development of study methods. Human CCs and NCs have different demands and thus different compositions and constructs are optimal. Hydrazone crosslinking was proven good option to synthetize hydrogels in RT.

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3D Printing of Bioresorbable Polymers for Personalized Medical Implants

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Introduction

Over the last three decades, the use of bioresorbable implants in orthopedic surgery has continued to gain importance. Their production with 3D printing technologies facilitates the preparation of individualized shapes, which enhances opportunities to further personalize medical implants [1] and to expand into other complementary application areas. To-date, one of the key challenges has been to identify and provide bioresorbable polymers that are suitable for 3D printing technologies. In this study, various bioresorbable RESOMER® polymers have been processed via common 3D printing technologies. The resulting samples were characterized and compared to injection molding products.

Experimental Methods

Three 3D printing technologies were applied using bioresorbable polymers (all from Evonik Nutrition & Care GmbH, Germany) provided in different forms:
(1) **Fused Filament Fabrication (FFF):** Filaments (1.75 mm diameter) were prepared using Poly(L-lactide), Poly(L-lactide-co-D,L-lactide), Poly(L-lactide-co-glycolide, Polycapro-lactone, Poly-dioxanone.
(2) **Selective Laser Sintering (SLS)**: Powder (D50 ~ 50 µm) was prepared using Polycaprolactone.
(3) **Freeformer Technology** (Arburg GmbH, Germany): done with granules of Poly(L-lactide-co-D,L-lactide).
The printed samples were compared to injection molded samples with respect to tensile strength and Young's modulus.

Results and Discussion

All applied technologies provided 3D printed samples in high quality. In comparison to the corresponding injection molded devices, the 3D printed samples displayed comparable mechanical stability, shown by tensile stress values (Fig. 1) and Young's modulus.

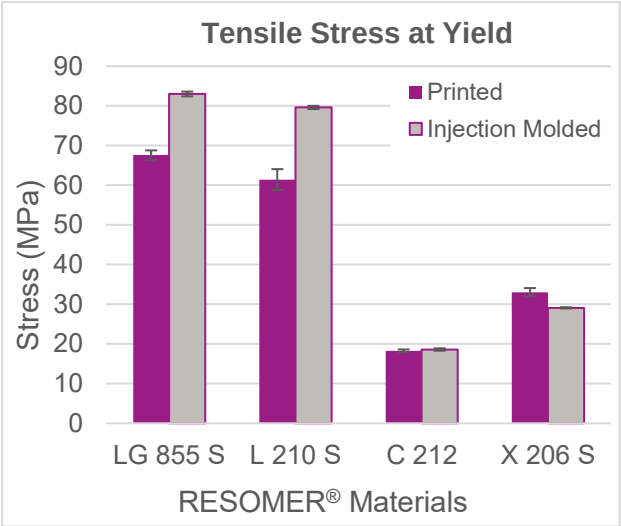

Figure 1. Tensile stress at yield values comparing samples prepared via FFF and injection molding

Conclusions

RESOMER® bioresorbable polymers can be used to prepare personalized implants. Due to their availability in various grades, the polymers can be efficiently processed with all common 3D printing technologies to encourage higher production flexibility.

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Tribological Model System Testing in Medical Engineering

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Introduction

Tribology is the science of interacting surfaces in relative motion and includes friction, wear and lubrication. Tribology has a long tradition in the fields of automotive and lubrication engineering. Over the past years, tribological approaches gained increasing importance in life science applications [1]. Advances in material development, increasing consumer demands and also the high requirements in terms of product safety, for instance due to FDA or MDR regulations, can be considered relevant motives for gaining an extensive understanding of material behavior under real life conditions. Examples of tribosystems related to the human body are shown in Fig. 1.

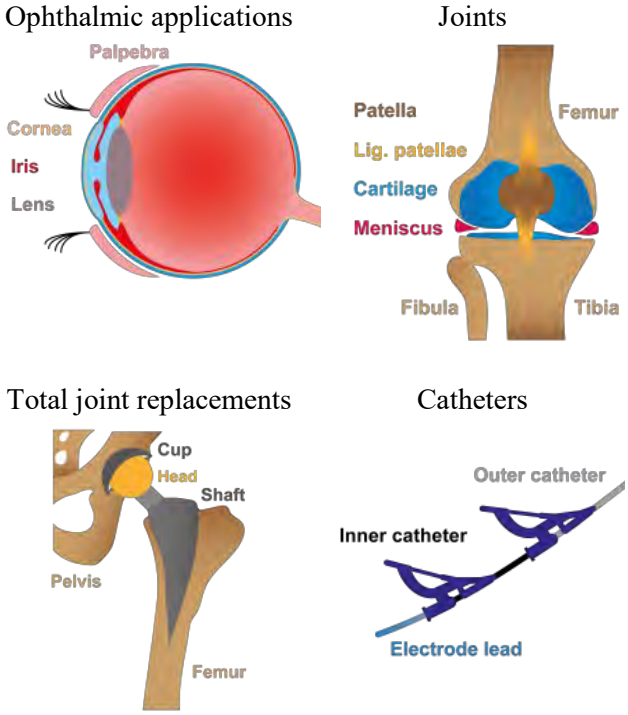

Figure 1. Example tribosystems in human body with relevance for medical engineering.

These tribosystems are characterized by a complex structure (e.g. surface and fluid structure) and by complex motion dynamics [2]. To mimic the real world conditions, the choice of surrogate specimen becomes crucial. Examples are:

- biological tissue such as cartilage
- biomimetic materials such as soft elastomers like Polydimethylsiloxane (PDMS) or artificial skin
- materials for medical device such as Polyether block amides [3], Polyvinyl chloride (PVC), or Ultra-high-molecular-weight polyethylene UHMWPE

This study deals with how to encounter the requirements of tribological model system testing with biological, bio-inspired, and artificial materials.

Experimental Methods

The main concept behind tribological model system testing is to simulate real life conditions to the extent possible. Beside the choice of specimen, test parameters such as contact pressure, speed, temperature or relative humidity, are also very important. Biotribological contacts generally experience relatively low speeds and low contact pressures, which can be simulated extremely well on the MCR Tribometer. Measurements are carried out at loads as low as 1 N and speeds ranging from several nanometers per second up to a few centimeters per second and thereby covering the most relevant speeds. We also present how one can use special adapters (see Fig. 2) for accommodating the above mentioned specimen

Figure 2. Left: Adapter with urinary catheter specimen and grub screw. Right: Urinary catheter specimen fixed in the adapter.

Results and Discussion

Results from tribological measurements are presented here in the form of breakaway torque measurements and extended Stribeck curves. Extended Stribeck curves cover the static and kinetic regime of friction and the transition between them [4]. Results from measurements with urinary catheter specimen and artificial skin are shown in Fig. 3. Each curve represents a single repetition with a new portion of sample. It can be seen that the limiting friction (the friction factor corresponding to the transition point) is reduced by around 25 % when a lubricant is used instead of physiological solution. Also, at elevated sliding velocities (v_s > 0.01 m/s), the friction factor for the catheter and artificial skin tribopair is lower, when a lubricant is used.

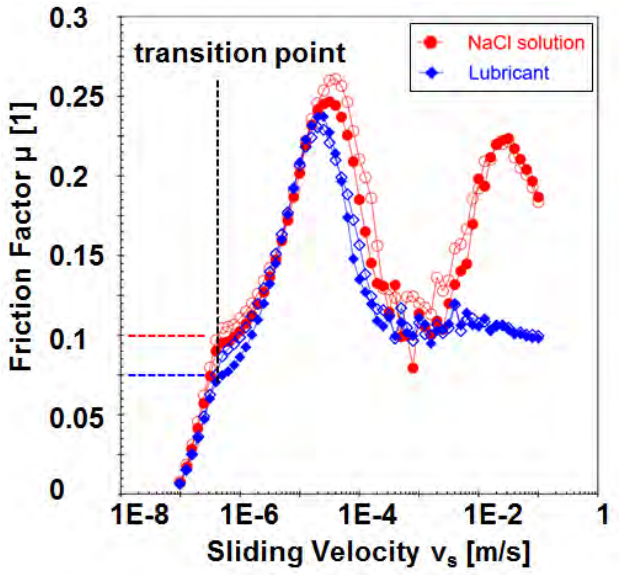

Figure 3. Extend Stribeck curves for urinary catheter vs. artificial skin tribopair, lubricated with physiological solution and a lubricant.

The contribution also covers different case studies in tribological model system testing, ranging from ophthalmic applications to orthopaedics.

Conclusions

Tribological model system testing enables for a deepened understanding of how materials being used in medical engineering perform when the entire system behavior is tested. The above mentioned characteristics of a tribosystem can be relevant to understand how a medical product may behave under real world conditions. Results from tribological testing can be helpful to distinguish which product would be more suitable for a certain application, such as low friction and low limiting friction, to reduce damages of human tissues. However, model system testing always has a certain level of abstraction and hence, critical evaluation of the data is essential.

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Multi-material Polymer Fibers for Medicine

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Introduction

With the recent progress on materials and processes associated with polymer fibers, advanced fiber-based devices are envisioned to be promising candidates for the next generation of optical probes for sensing, monitoring and minimally invasive surgical tools[43]. They can also serve as scaffolds, form bioengineered tissues, act as active releasing components in sutures or wound healing bandages, and form sensing fabrics for soft prostheses or the monitoring of physiological parameters. Electronic and optical fibers can also form a variety of one-, two-, and three-dimensional sensing networks such as those integrated in robotics or within structures and composites for health monitoring. Fibers also have the potential to constitute the next generation of smart textiles in a variety of medical applications. Among the different fabrication approaches of smart fibers, thermal drawing allow for a variety of fiber architectures and multi-materials structures. In this invited contribution, we will discuss the materials and processes at play to realize complex multi-material fiber geometries with advanced functionalities. We will discuss a few applications highly relevant for Medicine in using fibers as scaffolds, for pressure sensing, imaging, and controlled release.

Experimental Methods

The technique we used to fabricate multi-material fibers is thermal drawing[1-4]. After a preform is fabricated by assembling different materials at prescribed positions, it is placed in a furnace that softens the material that are pulled to form a thin fiber. A schematic of a drawing tower is shown in Figure 1a. This top-down process has various advantages. First, the architecture of the structure is fabricated in the preform at a macroscopic scale, which allows for flexibility in the desired architecture at the fiber level. Second, this process is scalable and allows for the fabrication of extended fiber length. Third, a variety of materials have been found to be compatible with thermal drawing in recent years. can be thermally drawn

Results and Discussion

In Figure 1b to 1d we show a few examples of fiber cross-sections we could create with this approach. Figure 1b shows a polymer fiber with a textured inner surface that can be used as regenerative scaffold[5]. A flexible fiber with bendable domains is shown in Figure 1c that can sense and localize touch[6]. This novel sensing concept functions as a one dimensional switch, with a drop of potential that depends on where the pressure is applied away from the contact points. These types of fiber sensors can be integrated in medical textiles and soft prosthesis for a variety of applications where high pressure points must be localized.

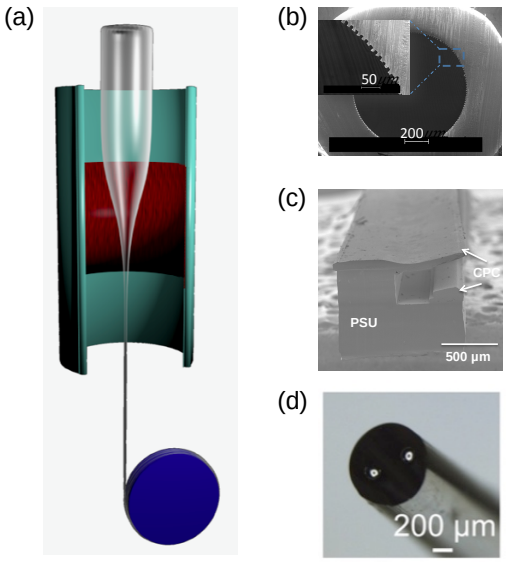

Figure 1. a) Schematic of the thermal drawing process. b) A textured thermally drawn polymer fiber. c) A polymer fiber with bendable polymer composite domains capable of touch sensing and localization. d) A soft elastomeric fiber with two liquid-metal electordes for deformation sensing.

In Figure 1d, we show a soft elastomeric fiber integrating two liquid metallic electrodes[7] that can act as a precise and robust mechanical deformation sensor.
The ability to draw soft step-index optical fibers[8] and fiber integrating Bragg mirrors[4,9] are also important developments. Recently, the ability to thermally draw natural polymers such as gelatin also opens exciting applications for sensing, monitoring and controlled release in health care.

Conclusions

The field of multi-material fibers is opening a breadth of new opportunities in health care and medicine. The understanding of the physical processes at play during the processing of a variety of polymers and polymer composite, as well as the design of novel sensing concepts, are key to further develop this promising technology.

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Fiber-Reinforced Composites for Implant Applications

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Introduction

Although metals, ceramics and particulate filler resin composites have successfully been used as implant biomaterials for decades, devices made out of these materials do not meet all surgical requirements. This presentation describes the structure and mechanism of function of biostable glass fiber reinforced composite (FRC) implants, which contains bioactive glass. Metal objects may interfere with some medical imaging systems (cone-beam computer tomography, magnetic resonance imaging) (1) and biomechanical mismatch of implant and bone may cause stress-shielding related bone resorption. There has been a lot of development in the field of composite biomaterials and bioactive materials and more focus of implant development has been put to biostable composites as implant material. Biostability of implantable medical devices is important to ensure success of the treatment in short and long term especially in load bearing applications.

FRC with continuous glass fibers in a biostable thermoset resin matrix provide high strength and high toughness non-metallic biomaterial. By adding bioactive glass (2) to the FRC implant, the implant supports osteogenesis and vascularization, and provide even antimicrobial properties for the implant (Figure 1) (3-5). Although the FRC implants and the material are used clinically in cranioplasties, further research is ongoing to demonstrate the most suitable implant designs for load-bearing applications for jaw bone reconstructions and orthopaedics.

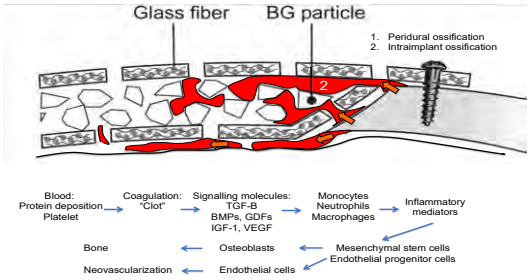

Figure 1. Schematic representation of the function of the FRC-bioactive glass implant

Conclusions

Use of glass fiber-reinforced composite as biostable skeleton for particles of bioactive glass has proved to the a material combination of choice for cranial implantology.

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Acknowledgments

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Engineering Bioactive Functional Polymers for Long-Term Therapies

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Engineering bioactive functional polymers to address medical challenges and needs including sites targeting considered an attractive approach in modern medicine. Medicinal polymers typically synthesized by anchoring bioactive functional moieties in their polymeric backbone. Here, the talk will focus on functional polymers engineering for medical implants were few examples will be presented and the functional moieties design will be elaborated. Implantable medical devices such as medical implants, cells encapsulation systems and sensors (e.g. CGMs) have revolutionized modern medicine and treatment approaches; however, their benefits and long-term use are undermined by microbial infection and immune-mediated foreign body response (FBR) leading to their failure. As such, there is a critical need not only for targeted, localized involvement, but also for extended long-term effect. The synthesis of variety of chemical modifications and engineering functionalized bioactive polymers based modifications for implants, cells encapsulation and for CGM sensor's coatings were the chemistry and structure-activity relationships will be presented.

The Visco-Elasticity of 2D Protein Networks – Applications for Stem Cell Expansion

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Introduction

The mechanical behaviour of the extracellular matrix has an important impact on cell phenotype. Although the mechanics of the ECM regulates a wide range of phenotypes, we recently reported the surprising observation that cells can adhere, spread and proliferate at the surface of liquids[1-3]. This observation is particularly surprising as the reinforcement of cell adhesion is thought to require a solid elastic or viscoelastic substrate that can resist cell-mediated contractile forces. We observed the formation of protein nanosheets, self-assembled at the liquid-liquid interface, displaying strong mechanical properties providing a suitable scaffold to promote cell adhesion and expansion. We showed that this is sufficient to regulate stem cell phenotype. However, the parameters controlling the self-assembly and the mechanical properties of protein nanosheets remain poorly understood. In this work we investigate the assembly of polymers and proteins at liquid-liquid interfaces, and the impact of pro-surfactants with a wide range of chemistries. We identify structural features that control the visco-elastic properties of the resulting nanosheets and regulate associated cell phenotype.

Experimental Methods

Assembly at liquid-liquid interfaces is studied using interfacial rheology. Protein nanosheets are characterised by scanning electron microscopy, atomic force microscopy and X-ray photoelectron spectroscopy. Cell adhesion and phenotype was characterised by fluorescence microscopy and qPCR.

Results and Discussion

In this work, we show the importance of parameters such as pH and concentration on protein self-assembly and the impact it has on interfacial mechanics. Importantly, we demonstrate the impact that pro-surfactant-protein interactions play on regulating the assembly and the interfacial mechanical properties of the corresponding interfaces. In addition, we show how these parameters regulate interfacial viscoelasticity and ultimately regulate cell adhesion and proliferation. Finally, we demonstrate the proof-of-concept of using such liquid substrates, in the form of emulsions, for stem cell culture in 3D bioreactors, and their simple recovery by centrifugation.

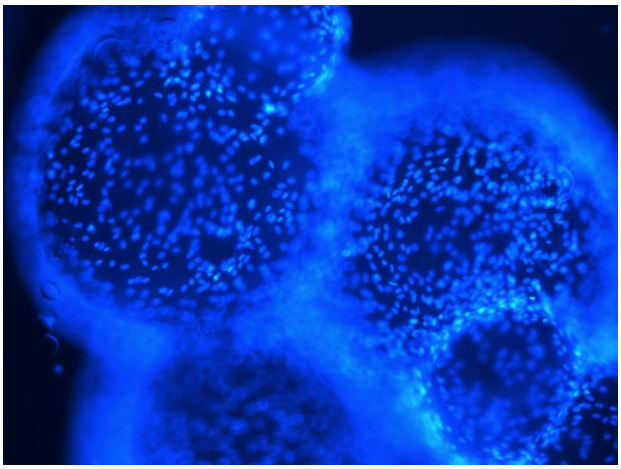

Figure 1. Mesenchymal stem cells cultured on emulsion droplets.

Conclusions

Overall, our results suggest that nanoscale mechanical properties of biomaterials may dominate over bulk physical properties. This concept has important implications for the design of biomaterials in the field of regenerative medicine and allow the rational design of liquid substrates for tissue engineering.

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Clinical investigation of the suitability of wood based nanofibrillar cellulose for skin graft donor site treatment

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[&]Co-last responsibility

ABSTRACT

BACKGROUND: Split-thickness skin graft donor cause pain and discomfort and may have healing problems, especially in elderly patients. While different types of dressings exist, there is no consensus regarding optimal dressing type to promote healing, reduce pain and improve patients' comfort. This prospective, single center clinical trial aimed to evaluate the qualities of nanofibrillar cellulose (NFC) wound dressing in split-thickness skin graft donor sites

METHODS:. NCF was compared with Suprathel®, widely used lactocapromer based wound dressing in Europe. We hypothesized that treatment with NFC wound dressing with a registered trade name of FibDex® would provide optimal donor site healing and that the healing time would be comparable with that of the Suprathel®. Twenty-four patients requiring skin grafting with mean age of 49 ±18, in the range of 21-74 years, were enrolled in the investigation performed at Helsinki Burn Centre. Principal outcome measure was wound healing time, which was assessed by self-detachment of the wound dressings. As secondary outcomes the percentage of epithelialization was measured as well as subjective pain using Visual Analogue Scale (VAS) scale. Also, the the scar quality was assessed with Patient and Observer Scar Assessment Scale (POSAS) and the characteristics of the epithelialized skin, including elasticity and transepidermal water loss (TEWL) were measured quantitatively using DermaLab® Skinlab COMBO at 1 and 6 months post-operatively.

RESULTS: We observed no statistical differences between NFC dressing (FibDex®) and Suprathel® regarding wound healing time, epithelialization, or experience of pain on donor sites. The mean healing time for FibDex (N=24) was 18.5 (±5.3) post-operative days (POD) and for Suprathel® (N=16) it was 18.5 (±4.6) POD (p=0.857). The modified Patient and Observer Scar Assessment Scale

(POSAS), in which the lower the value the better is the treatment, was utilized to follow the healing of the skin graft donor site. The obtained overall values for the Observer were at 1 month 2,81 for FibDex and 2,85 for Suprathel® and those for 6 months were 2.19 for FibDex and 2.31 for Suprathel®. For the Patient scale the values were at one month for FibDex 5,15 and for Suprathel® 4,92 and at 6 months for FibDex 3,00 and 3,58 for Suprathel®.

Skin characteristics measured using DermaLab® showed statistically significant improvement of skin elasticity at one month after treatment with FibDex compared with Suprathel® in terms of viscoelasticity ($p=0.016$) and elastic modulus ($p=0.010$). The values of transepidermal water loss (TEWL) and elastic modulus in FibDex- or Suprathel®-treated donor site skin at 1 month differed significantly from the values of the healthy skin. When comparing the values of one month and six months, a significant improvement was observed with NFC dressing regarding TEWL ($p=0.0004$) and with Suprathel® regarding TEWL ($p=0.010$), viscoelasticity ($p= 0.041$) and elastic modulus ($p=0.047$) between the time points, and at six months all the values were in order of the healthy skin.

CONCLUSIONS: FibDex provides efficient wound healing at skin graft donor sites and is comparable to Suprathel® in terms of wound healing time, epithelialization and pain. According to DermaLab® measurements, the elasticity of the scar is significantly improved with Fibdex compared to Suprathel®. Further, the POSAS results of the scar appearance prefers the use of FibDex over Suprathel® for skin graft donor site treatment.

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Poster Abstracts

pH-Sensitive Drug Release from Photo-crosslinked Poly(ester-anhydride) Networks

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Introduction

Poly(ester-anhydride)s degrade fast through surface erosion, which is usually considered a benefit in drug release applications. Previously, ε-caprolactone-based crosslinkable poly(ester-anhydrides) have been studied in vitro and in vivo, and no cytotoxicity and no or only mild inflammatory reactions in mice and rats were observed.[1]

Degradation of polyanhydrides is pH-sensitive; they are more stable in acidic conditions and degrade faster as the pH value is increasing.[2] Therefore, possible application area for these fast degrading poly(ester-anhydride)s in oral drug delivery. The hypothesis is, that the polymer matrix would protect the drug in the acidic conditions in the stomach until it reaches conditions near neutral pH in the intestine. In this study, effect of pH-condition on hydrolysis of the polymer and drug release of two different model drugs were studied.

Experimental Methods

The precursor was synthetized by a similar method as used earlier[3]. Briefly, the ε-caprolactone based hydroxyl-terminated oligomers was synthesized in a batch reactor by using 10 mol-% trimetholylpropane as a co-initiator and 0.02 mol-% Sn(II)octoate as a catalyst. Subsequently, oligomers were functionalized with succinic anhydride to obtain acid-terminated oligomers. Crosslinkable precursors with labile anhydride bonds were obtained by allowing the acid-terminated oligomer to react with methacrylic anhydride. Reactions were monitored with nuclear magnetic resonance spectrometer.

Model drugs (vitamin B12 and lidocaine) and photoinitiator TPO (5 wt-%) were mixed with the precursor until homogeneity was achieved. A teflon mould was used to obtain discoids (2 mm thickness, 2 mm in diameter, 9 mg in weight). Crosslinking was carried out at room temperature by photoinitiaton in visible light for 18 minutes. Hydrolysis and drug release were studied in phosphate buffer solution (pH 2.1, 6.8, 7.4 and 12). The amount of released drug was analysed with UV/Vis-spectrophotometry.

Results and Discussion

The hydrolytic degradation of poly(ester-anhydride) in different pH conditions is presented in Figure 1. Within 24 h, only 10% of sample was degraded in pH 2.1, whereas samples in other conditions were completely eroded. Drug release studies of water soluble model drugs vitamin B12 (1355g/mol) and lidocaine (234g/mol) showed that the drug release was greatly affected by the size of drug molecule. In acidic conditions, around 50% of lidocaine was released in 24h, whereas less than 10% of vitamin B12 was released. Therefore, it appears that lidocaine is released through diffusion and polymer degradation, whereas B12 is released only through polymer degradation.

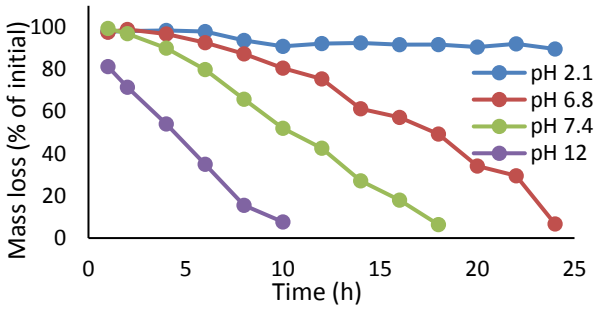

Figure 1. Mass loss of ε-caprolactone-based poly(ester-anhydride) samples in different pH conditions.

Conclusions

The hydrolytic degradation of poly(ester-anhydride) samples is highly pH sensitive. The degradation rate is slow at acidic conditions, and increases as the pH-value of buffer solution is higher.

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Acknowledgments

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3D printed capsules for sustained drug release from nanocellulose hydrogel

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Introduction

3D printing is an effective method for fast prototyping of pharmaceutical tablets and devices with unique shapes and structures enabling new types of controlled release. In this study, we combined this versatility with anionic nanocellulose hydrogel whose versatile drug release capabilities have been demonstrated for sustained release applications [1].

Experimental Methods

Non-active capsules were manufactured via 3D printing from biocompatible and biodegradable poly(lactic acid) (PLA) with designed inner cavities. As a novel method, the capsules were filled with a drug suspension composed of model compounds and anionic cellulose nanofiber (CNF) hydrogel, and the drug release was measured for three weeks *in vitro*.

Results and Discussion

The main benefit of this device is that the release of any CNF-compatible drug can be modulated simply by changing the geometry of the PLA capsule (Fig 1)

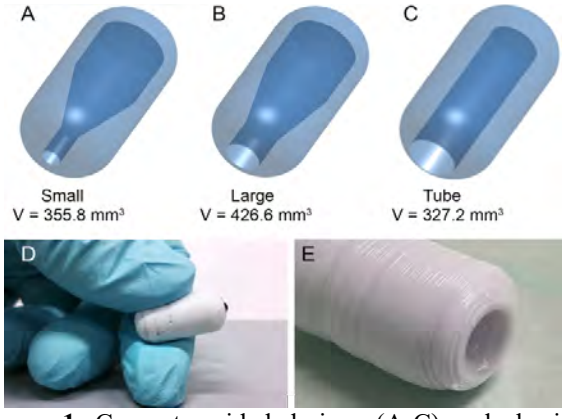

Figure 1. Computer aided designs (A-C) and physical printed versions (D-E) of the studied PLA capsules.

After optimizing the size and shape of the capsules inner cavity, drug release tests were performed with common beta blockers metoprolol and nadolol as the model compounds (Fig 2).

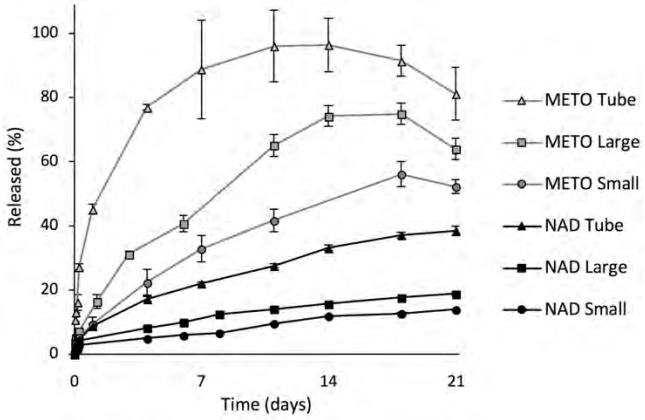

Figure 2. Scaled cumulative release of the model compounds metoprolol (METO) and nadolol (NAD) from the three capsule designs (Tube, Large, and Small) carrying anionic cellulose nanofiber hydrogel drug formulations (mean ± S.D., n = 3). The experiments were conducted at 37 °C in DPBS buffer.

Conclusions

The final results demonstrate that the sustained release profiles provided by the CNF matrix can be controlled via the geometry of the 3D printed PLA capsule, resulting in adjustable sustained release for the model compounds for up to three weeks *in vitro*.

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Acknowledgments

The authors acknowledge and thank the University of Helsinki for co-operation and for providing access to their laboratories and screening instrumentation.

Well-defined Telechelic Oliogodepsipeptides as Precursor Materials for Nanoparticulate Gene Carrier Systems

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Introduction

Well-defined telechelic oligodepsipeptide oDPs are the prerequisite to create the ABA triblockcopolymers by sequential copolymer synthesis. The most commonly used catalyst for the polymerization of MDs as well as their copolymerization with other cyclic monomers i.e. dilactides and ε-caprolactone is tin(II)-2-ethylhexanoate (Sn(Oct)2). An alternative choice as catalyst for the ROP of lactone based monomers is 1,1,6,6-tetra-*n*-butyl-1,6-distanna-2,5,7,10-tetraoxacyclodecane (Sn(IV) alkoxide), as demonstrated for the ROP of L-dilactide, 1,5-dioxepan-2-one or ε-caprolactone. We hypothesized that Sn(IV) alkoxide catalyzed ROP allows a more precise control of the ROP reaction and result in well-defined telechelic oDPs.

Experimental Methods

3-sec-butyl-morpholine-2,5-dione (BMD) was synthesized according to the procedures described in references.[1,2] MDs (10 mmol) were polymerized in pre-silanized 10 mL Schlenk tubes using ethylene glycol as initiator and either $Sn(Oct)_2$ or Sn(IV) alkoxide as catalyst. The degree of polymerization (DP) was set to 60 and the molar catalyst:monomer ratio was set to 1:100.

Matrix Assisted Laser Desorption/Ionization-Time Of Flight Mass Spectroscopy (MALDI-TOF MS) was performed on a Biflex III spectrometer (Bruker Daltonik, Leipzig, Germany).

Results and Discussion

MALDI-TOF MS of the obtained oligomers was performed to characterize the terminal groups. Five series of signals corresponding to four different species could be identified as shown in Figure 1a. Two series (3 and 4) correspond to telechelic oligomers doped by sodium and potassium as expected. Series 2 with a mass difference of 44 in relation to series 4 could originate from water initiated polymerization. Potential explanation for this water initiated polymerization could be the hydrophilic nature of both Sn(Oct)2 and EG despite of carful drying of reactants and inert condition during the polymerization reaction.

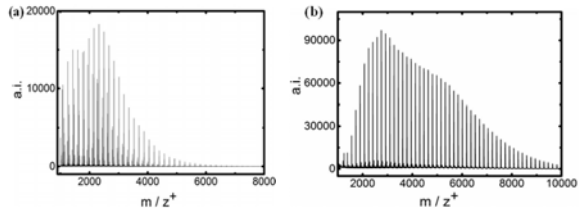

Figure 1. MALDI-TOF MS of oligomers based on BMD. Catalyst/initiator used: (a) Sn(Oct)2/EG; (b) Sn(IV) alkoxide.

Conclusions

Thanks to the well-defined telechelic character of the synthesized oDPs they resemble interesting building blocks for subsequent postfunctionalization like nanoparticulate gene carrier systems.[2]

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Pectin aerogels for drug delivery

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Introduction

Aerogels are open-pores lightweight and nanostructured materials with high specific surface area; they are thus very promising materials to be used as matrices for loading and release of active compounds. *Bio*-aerogels are based on polysaccharides which are biodegradable and biocompatible; their synthesis does not involve any toxic component which opens many ways of using them in food, cosmetics, pharma and biomedical applications. The goal of the work was to demonstrate how to tune the morphology and properties of pectin aerogels to vary release kinetics of model drug, theophylline.

Experimental Methods

Aerogels were prepared from low methylated pectin via dissolution, gelation (or non-solvent induced phase separation) and drying with supercritical CO_2. The release of theophylline was monitored using UV-vis spectrophotometer.

Results and Discussion

Polymer concentration, solution pH and presence of divalent ions were varied to tune pectin gelation mechanism and the state of matter (solution or gel) before solvent exchange. Aerogel density varied from 0.05 to 0.25 g/cm^3 and specific surface area from 300 to 600 m^2/g. Some examples of aerogel morphology are shown in Figure 1.

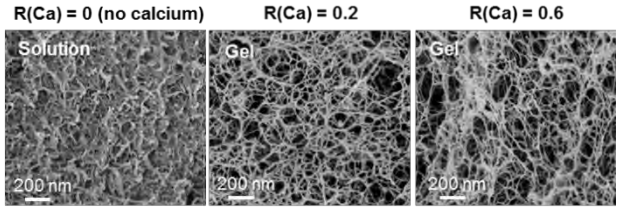

Figure 1. Examples of morphology of pectin aerogels obtained from solution (no calcium added) and gels (calcium added at different molar ratios)

Theophylline was loaded in pectin matrix via impregnation in ethanol and drug *in vitro* release was studied in simulated gastro-intestinal fluids. The influence of the preparation conditions on pectin aerogel density, specific surface area, morphology and drug release kinetics will be discussed. Organic-organic (pectin-cellulose) and organic-inorganic (pectin-silica) composite aerogels were also prepared. This allowed a significant variation of release mechanisms and its kinetics, from 3-5 to 10-15 hours.

Conclusions

We demonstrated that pectin aerogels are versatile carriers for drug delivery.

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Ultrafast *In Situ* Forming Poly(ethylene glycol)-Poly(amido amine) Hydrogels with Tunable Drug Release Properties via Controllable Degradation Rates

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Introduction

Dendrimers have attracted increasing attention for the preparation of biomedical hydrogels thanks to their uniformity combined with control over their size, architecture, density and surface groups.[1] In most poly(amino amide) (PAMAM) based hydrogels, linear poly(ethylene glycol) (PEG) was employed as crosslinking agent. However, star-shaped PEGs offer various advantages over linear PEGs, such as a higher concentration of end groups, which may result in faster gelation. Furthermore, control over hydrogel degradation is an important item that has yet received little attention regarding PEG-PAMAM hydrogels. This prompted us to prepare *in situ* forming PEG-PAMAM hydrogels by reacting PAMAM with multi-armed PEGs containing either a hydrolysable ester group or a stable amide group near each PEG end.

Experimental Methods

Hydrogels were prepared in NaH_2PO_4/Na_2HPO_4 buffer (pH 8) via the amidation reaction between N-succinimidyl ester (NHS) end groups of multi-armed PEG and amino end groups of PAMAM generation 2.0. To control the properties of the PEG-PAMAM hydrogels, PEGs were used with different arm numbers (4 or 8) as well as different linkers (amide or ester) between the PEG arms and their terminal NHS groups (Figure 1).

Results and Discussion

Rheology measurements showed that the hydrogels form within seconds after mixing the PEG and PAMAM precursor solutions. The storage moduli increased with crosslink density and reached values up to 2.3 kPa for hydrogels based on 4-armed PEG. Gravimetrical degradation experiments demonstrated that hydrogels with ester linkages between PEG and PAMAM degrade within 2 days, whereas amide-linked hydrogels were stable for several months. The release of the model drug fluorescein isothiocyanate-dextran ($4 \cdot 10^3$ or $2 \cdot 10^6$ g/mol, FITC-DEX4K and FITC-DEX2000K, respectively) from amide-linked hydrogels was characterized by an initial burst followed by diffusion-controlled release, of which the rate depended on the size of the drug. In contrast, the release of FITC-DEX2000K from ester-containing hydrogels was governed mainly by degradation of the hydrogels and could be modulated via the ratio between ester and amide linkages. Cytotoxicity experiments showed that the PEG-PAMAM hydrogels are non-toxic to mouse fibroblasts.

Conclusions

The possibility to be formed *in situ* and their tunable mechanical, degradation and release properties make these PEG-PAMAM hydrogels appealing as controlled drug delivery systems.

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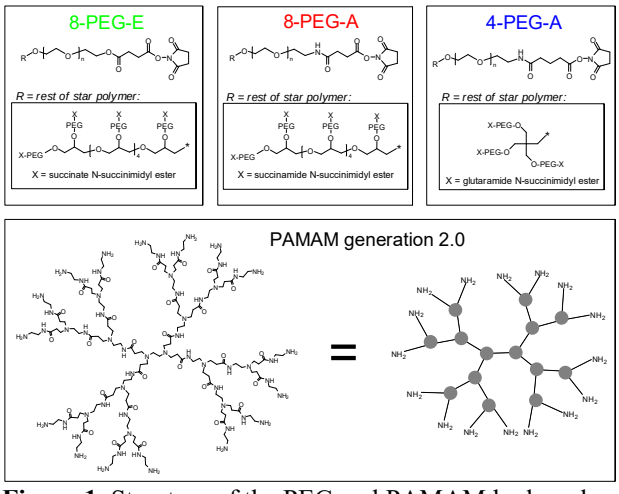

Figure 1. Structure of the PEG and PAMAM hydrogel precursors used in this study.

Degradable polyphosphoester networks: an efficient chemical cross-linking under mild conditions

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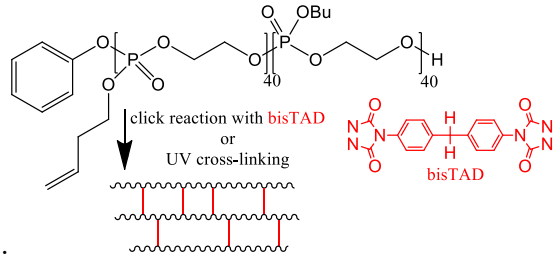
Introduction

The design of implants and scaffolds for tissue engineering often requires biodegradable and biocompatible materials. In this respect, polyphosphoesters[1] (PPE) are an emerging class of degradable materials with promising biomedical applications since the pentavalency of phosphorus atom allows easy tuning of physico-chemical properties. Mostly, PPEs are amorphous rubber and therefore cross-linking is required to bring about appropriate mechanical properties. In this work, an unsaturated alkene side-chain was introduced on the cyclic phosphate monomer according to a one-step reaction followed by its organocatalyzed copolymerization with methyl- or butyl-cyclic phosphoester comonomers. These unsaturations are then used for cross-linking at room temperature, without side-product formation by using an Alder-ene click reaction (scheme 1). The as-obtained networks are characterized by swelling experiments and their hydrolytic degradation profiles are compared to networks obtained by UV irradiation of the same unsaturated precursor.

Scheme 1. Synthesis strategy for the PPE networks



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Experimental Methods

The unsaturated copolyphosphoester is obtained by organocatalyzed ring-opening polymerization following a procedure described in reference 2. The click reaction cross-linking is performed by adding a solution of bis-TAD in THF to a solution of unsaturated copolyphosphoester in CH_2Cl_2 at room temperature while the UV cross-linking is achieved in presence of Irgacure as photosensitizer with a light wavelength of 360 nm.

Results and Discussion

After cross-linking, the starting oily material leads to elastomeric easily handling materials (Figure 1). For UV crosslinking, the sample is limited to thin films for the reaction to be efficient while by the click reaction, there is no limitation on the samples thickness.

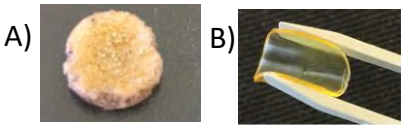

Figure 1. Picture of PPEs networks obtained by: A) click reaction with bisTAD B) UV cross-linking.

Conclusions

The innovative click reaction cross-linking strategy by adding bis-TAD to an unsaturated PPE allows to prepare degradable PPE networks in mild conditions. This strategy exhibits the advantage to avoid the use of UV light remaining thus appropriate for thick samples not fully transparent.

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An investigation of novel GPTMS-cross-linked Pectin-based sponges as sustainable scaffolding strategy for applications in tissue engineering

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Introduction

Renewable biomaterials are gaining a great interest for the sustainable and bioactive scaffolds Tissue Engineering (TE) applications [1]. Pectin is a complex structural polysaccharide which represents one of the most abundant renewable polymers [2]. Recently, pectins emerged as novel and potential candidate as TE-based biomaterials due to their biocompatibility and non-toxicity [3]. The present study explores the use of novel pectin-based sponge chemically crosslinked by (3-Glycidyloxypropyl) trimethoxysilane (GPTMS) for guiding the regeneration of neo-tissues. The effect of altering the GPTMS concentration is evaluated in terms of structures' mechanical and swelling properties.

Experimental Methods

Low methoxyl (LM) citrus pectin (Classic CU 701, Herbstreith&Fox) was purified as described in [4] and dissolved in ultrapure water at 70°C to obtain a 4% (w/v) solution. GPTMS was added to the purified pectin (pPEC) solution at 1.5 %, 0.98%, 0.49%, 0.24%, 0.1 % (w/w) with respect to the pPEC content. After 40 min stirring, the resulting mixtures were poured into cylindric molds (13 mm diameter x 10 mm height) and cooled at 4°C for 3h and then at 20°C overnight. Finally, they were freeze-dried at 60°C for 24 h to obtain sponge-like porous structures. The swelling degree (SD) of the pPEC-sponges (n = 10) was evaluated in ultrapure water at 37°C at predefined time points for 96h as eq: $(W_s\text{-}W_0/W_0)$, where W_0 and W_s are the weight of the dry and swollen sample, respectively. The compressive Elastic moduli (E) of fully swollen pPEC- sponges (n = 10) with different GPTMS contents were assessed by uniaxial compressive tests conducted with a Z005 Zwick/Roell testing machine at strain rate of 1% s^{-1} up to 30% of deformation.

Results and Discussion

Figure 1 (a) shows the pPEC-sponges crosslinked with different amount of GPTMS. Higher concentrations of GPTMS resulted in a significant lowering of SD (Fig. 1 (b)) and increasing of E (Fig. 1 (c)) due to the growing cross-linked density of the polymer chains inside the structure [5].

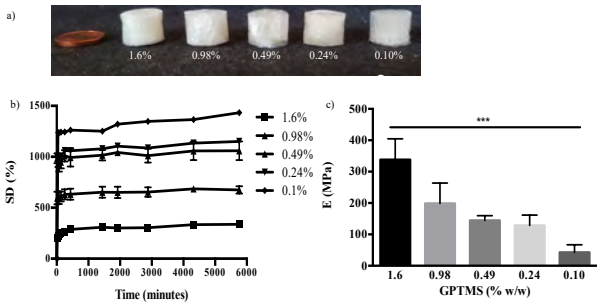

Figure 1. a) pPEC-sponges for different GPTMS concentration (% w/w). Effect of GPTMS concentration on SD and E of pPEC-sponges. (*) indicates a statistical significance ($p < 0.0002$) between the samples.

Conclusions

We proposed here novel , sustainable and chemically crosslinked pectin-based sponges for TE. We found that their mechanical and swelling properties can be tuned by simply varying the GPTMS concentration to tailor their for specific TE applications. Next steps will be focused on the investigation of their degradation, morphological and cytocompatibility properties as function of the GPTMS concentration.

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Inhibition of cellular senescence by polydopamine coating to maintain mesenchymal stem cells

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Introduction

Mesenchymal stem cells (MSCs) show great potential in cell therapies and regenerative medicine. However, the loss of stem cell properties due to cell senescence in long term *in vitro* expansion hinders the application of MSCs. The strategies to maintain self-renewal of stem cells include gene transduction and controlling the culturing environment such as hypoxia treatment and surface coating [1]. Polydopamine (PDA) provides strong binding sites for material surface and biomolecules, thereby supports a long term cultivation of stem cells [2]. Moreover, PDA coating may have the capacity to decline the oxidative stress due to its high oxygen affinity, which can reduce the intracellular ROS generation [3]. However, the influence of PDA coating on MSC behavior is not clear.

Experimental Methods

Dopamine solution (1 mg/ml) was prepared by dissolving dopamine in 50 mM Tris buffer (PH=8.5). Tissue culture plate (TCP) was coated with dopamine solution for 2 hours at 37 °C, then the plates were washed twice by PBS and cell culture medium (10% FBS in DMEM) once. Non-coated and polylysine coated TCPs were used as control. The human adipose derived stem cells (hADSCs) were seeded on the surfaces. Cell proliferation was determined using a Cell Counting Kit-8 (CCK-8). Cellular senescence was examined via β–galactosidase staining and using a cellular senescence assay kit. The expression of aging related genes was analyzed with PCR array. Statistical analysis was performed using the two tailed independent-samples *t* test, and $p<0.05$ was considered to be statistically significant.

Results and Discussion

The cells on all test surfaces (TCP, PDA and polylysine coated TCPs) showed similar morphology after 3 days of culture. The coating of PDA and polylysine improved the cell proliferation rate (Fig. 1A). Compared to non-coated TCP, cells on PDA coated TCP showed significantly decreased level of cellular senescence (Fig. 1B). Most of the aging related genes were down-regulated on PDA coated surface as compared to non-coated surface. The low

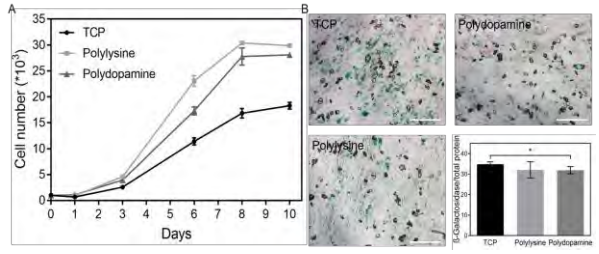

Figure 1. (A) The proliferation rate of hADSCs on different surfaces. (B) Staining and quantification of β–galactosidase after 2 weeks of cell culture, scale bar=200 μm, *p<0.05, n=6.

level of senescence on PDA coated surface might be caused by the reduced oxidative stress. In addition, PDA coating might lead to the absorption of functinal biomolecules on the material surface, which further regulated the attached hADSCs.

Conclusions

PDA coating could improve hADSC proliferation, maintain their self-renewal and reduce the cellular senescence. Our results suggested a potential strategy to modify cell culture materials for *in vitro* stem cell maintenance.

Acknowledgments

The work was supported by the Helmholtz Association (programme-oriented funding).

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Additive Manufacturing of Bone Regeneration Implants for Large Critical Size Defects

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Introduction

There is a major clinical need to replace and regenerate bone tissue due to trauma or disease. By using bioresorbable materials an implant can be engineered to replace the damaged tissue as long as needed. Eventually, the implant slowly degrades while being replaced by bone tissue. Combination of bioresorbable materials with 3D printing offers an engineering route to achieve individualized implants based on imaging data.

In bone grafting, utilization of biopolymers in composites with bioceramic β-tricalcium phosphate (β-TCP) show promising results due to their osteoconductive properties. Calcium phosphates are a major component of natural bone and β-TCP is a phase pure synthetic, resorbable and osteoconductive ceramic that is widely used in commercially approved products. Polymer composites containing 60wt% β-TCP have demonstrated to have similar osteogenic activity as pure β-TCP [1] .

We present 3D printing of large porous composite scaffolds of poly(trimethylene carbonate) (PTMC) including high amounts of β-tricalcium phosphate. Such composites show exciting properties for use as patient-specific osteoconductive and resorbable bone grafts.

Experimental Methods

Photocrosslinkable poly(trimethylene carbonate) (PTMC) was synthetized by ring opening polymerization of trimethylene carbonate and further functionalization with methacrylic anhydride. The PTMC macromer (Mn=9500g/mol) was mixed with osteoconductive β-TCP, non-reactive diluent propylene carbonate and a photoinitiator to create printable resins. By adding a die to the resin the thickness of the cured resin layers could be optimized. After initially printing small test samples the focus moved to optimizing the resin composition and printing parameters in order to manufacture large patient-specific prototypes.

Results and Discussion

Addition of β-TCP not only adds bioactivity but also improves the mechanical properties of the manufactured scaffolds. Following printing of small test specimen containing up to 60wt% β-TCP we also manufactured large patient-specific prototypes including 45wt% β-TCP (Figure 1.). SEM imaging shows that the osteoconductive β-TCP are readily available on the surface for instant initiation of bone regeneration. Parameters such as porosity, pore-size, scaffold architecture as a well as possible addition of growth factors can be optimized to create optimal bone regeneration environment. Following successful manufacturing of large implants, an *in vivo* study with a large critical size defect has been planned.

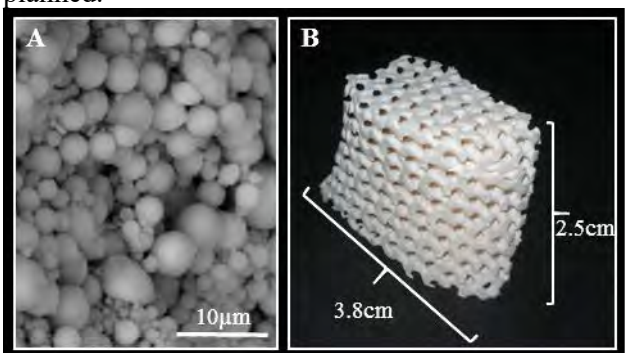

Figure 1. A. SEM image of composite scaffold showing spherical β-TCP particles on the surface. B. Large prototype of patient-specific bone regeneration scaffold.

Conclusions

The biocompatibility and bioresorption of PTMC coupled with the osteoconductivity of β-TCP forms an exciting bone regeneration scaffold especially when manufactured by high-resolution 3D printing.

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Acknowledgments

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Bio-compatible, photo cross-linkable polyurethane resin for manufacturing of 3D-printed nerve guidance conduits

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Introduction

Peripheral nerve damages have high risk of treatment failure and low regeneration rate. Autografting is one of the standard methods for repairing ruptured nerves. However, morbidity and limited number of the donor sites are associated with this technique [1]. An advantageous alternative for the autografts can be Nerve Guidance Conduits (NGC). However, the existing commercial NGCs aren't applicable for repairing the long gaps in major nerve ruptures. Furthermore, they need improvement in geometrical design to provide better environment for growing the nerve cells [2]. In this respect, using the advanced manufacturing methods such as 3D printing can provide more flexibility in producing precise and customized geometry [3].
In this study, a photo cross-linkable polyurethane resin with reactive acrylic groups was developed. The synthesized resin was used in stereolithography for manufacturing precise NGC with the 2mm internal diameter, 0.4mm wall thickness, and 20mm length. In addition, special geometrical features such as holes with the diameters of 0.2 mm and grooves with depth of 0.2 mm were applied for producing more effective NGC (Fig.1)

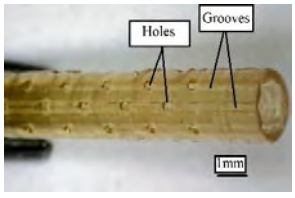

Figure 1. printed nerve guidance conduit

Experimental Methods

The block polyurethane (PUCL-EG) was synthesized using polycaprolactone diol (M_n 530 g/mol), poly ethyleneglycol (PEG, M_n 400 g/mol), hexamethylene diisocyanate (HDI, 98%), dibutyltindilaurate (DBTDL, 95%), hydroxyethyl methacrylate (HEMA). For printing the resin ethyl phenyl(2,4,6-trimethylbenzoyl)phosphinate (1.5 wt% relative to the macromer) and Orasol Orang G as dye were used.

Results and Discussion

Chemical structure of synthesized resin was analyzed by FTIR and NMR methods. The results indicate formation of acrylic ends groups. Furthermore, the mechanical and thermal analyses of the photo cured specimens show that the cured polyurethane resin has 140% tensile strain at break and 100% elastic recovery, which all completely meet mechanical and thermal criteria required for the NGC. Moreover, the Young modulus and glass transition temperature are 2.36 MPa and -26 °C respectively. Furthermore, in-vitro studies show the growth of Fibroblast cells on the surface of polymeric substrate and the Scanning Electron Microscopy (SEM) images support the results.

Conclusions

Synthesized PUCL-EG resin shows appropriate properties for producing NGC using 3D-printing technique. This enables us to enhance the NGCs functionalities by generating the accurate and modified geometrical features.

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Acknowledgments

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Thiol-Michael reactions of optically-active mercapto-acids in aqueous medium

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Introduction

Defined chemical reactions in physiological environments are a prerequisite for the in-situ formation of materials or implants with potentially to be used in drug delivery systems or for scaffolds. For this purpose, bioorthogonal reactions that are basically inert to the plethora of functionalities present in-vivo are highly beneficial to avoid impairing physiological processes. In this context, 'click' reactions like thiol-Michael reactions have been successfully employed [1-2]. Such typically high yielding and selective reactions are nonetheless subject to the individual stereo-electronic and physical properties of specific substrates thus require exact understanding to assist the selection of substrates for relevant applications.

Here, thiol-Michael click reactions of (*S*)-2-mercapto-carboxylic acid analogues of L-phenylalanine (SH-Phe) and L-leucine (SH-Leu) were studied. The chiral thio-analogues of hydrophobic amino acids are of interest as subunits of peptido-mimetic thioesters and their potential as substrates for probing certain biological cues.

Experimental Methods

In-situ ^{1}H-NMR was used to follow the reaction kinetics of the synthesized (*S*)-2-mercapto-carboxylic acids with diethylene glycol ethyl ether acrylate and methoxy polyethylene glycol maleimide (ca. 750 Da) as Michael acceptors.

Confirmation of reaction products was carried out with ^{13}C-NMR, electrospray ionization mass spectrometry (ESI-MS) and reverse phase high performance liquid chromatography (RP-HPLC).

Results and Discussion

The reactivity of the thiol substrates was studied in the presence/absence of trimethylamine (TEA) as catalyst and in varying molar equivalents at room temperature. Highest acrylate conversion for TEA-catalysed reactions with SH-Leu was (76 ± 4) % as determined from in-situ ^{1}H-NMR for 1.0 equivalent TEA after 480 s. In the absence of TEA, using preconditioned stock solutions of SH-Leu at pH 7.2 and 8.0, complete conversion was observed for the maleimide and acrylate respectively after 480 s. ESI-MS of the reaction mixtures with complete conversions, yielded ionization patterns consistent with the expected thiol-Michael product. ^{13}C-NMR and RP-HPLC were successfully used to confirm the presence of the thiol-Michael product as the sole compound and complete substrate conversion.

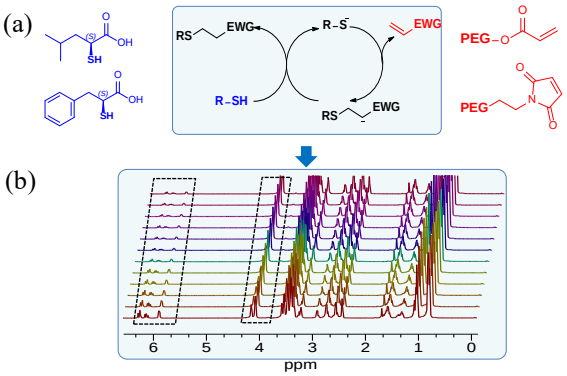

Figure 1. (a) Thiol-Michael reaction mechanism and chemical structures of reaction substrates (b) Representative stacked ^{1}H-NMR spectra recorded over 4 minute intervals.

Conclusions

Fast reaction rates and complete acrylate/maleimide conversion were only realized at pH 7.2 or higher suggesting the possible use of SH-Leu under physiological conditions for thiol-Michael reactions. No thiol-Michael product was observed for reactions with SH-Phe, thus highlighting that even structurally closely related compounds may have quite different reactivities.

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Poly(ε-caprolactone-co-p-dioxanone) copolymers: reaching the balance between processability and degradation rate

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Introduction

Polycaprolactone (PCL) is among the polymers most utilized for tissue engineering scaffolds 3D printing.[1] PCL has good viscoelastic properties and low melting temperature that render it suitable for additive manufacturing of scaffolds, but the resorption process of the PCL devices is very slow, up to 3-5 years for PCL of low molecular weight, M_n = 50 kg mol^{-1}. Our aim was to develop, by bulk copolymerization of CL with p-dioxanone (DX), copolymers suitable for the fabrication of scaffolds that could be processed in a manner similar to that for PCL, but degrade faster than PCL while maintaining a certain degree of crystallinity, a low T_m and high thermal stability.[2]

Experimental Methods

Random copolymerization of CL and DX were optimized using tin octanoate as catalyst. The reaction were performed in bulk at 130 °C. The copolymer composition and microstructure were elucidate through 1D and 2D NMR experiments. Molecular weight and thermal properties were characterized by SEC and DSC, respectively.
Films were prepared by casting solution of the copolymers in $CHCl_3$. Films were utilized for contact angle measurements, mechanical tests and hydrolytic degradation during 120 days.

Results and Discussion

High molecular weight copolymers consisting of CL and DX, with a content of DX up to 20 mol%, were prepared by bulk copolymerization (Figure 1).

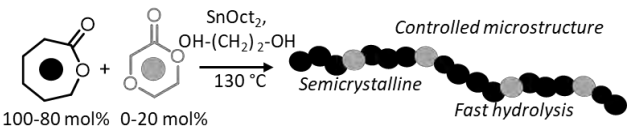

Figure 1. Random copolymerization of CL and DX.

The copolymer microstructure was elucidated by NMR characterization and consisted of long CL segments split by isolated DX unit. The CL segments had molecular weight below 3 kg mol^{-1}. PCL oligomers that have molecular weight below this value, are soluble in the aqueous medium and can diffuse out of the matrix, undergo intracellular resorption and lead to erosion of the implant.
CL/DX copolymers were semicrystalline with a crystalline lattice typical of PCL, and the melting point was function of the amount of DX in the copolymer. High thermal stability was assessed for all the compositions.
The copolymer films were more elastic and softer than PCL. The beneficial effect of the DX units on the PCL hydrophobicity and slow degradation kinetics was also evaluated. A content of of 15-20 mol% of DX allowed to reach the limiting molecular weight of 3 kg mol^{-1} in 8-9 months, thus, much faster than the 3 years required for a PCL with M_n of 50 kg mol^{-1}.[2]

Conclusions

A strategy was developed to obtain degradable and random copolymers of CL and DX, which represent suitable materials for the 3D printing of tissue engineering scaffolds. In fact, such materials combine the good processability and thermal stability of PCL with the hydrophilicity, good degradability and faster degradation of poly(p-dioxanone), PDX. Despite the low melting point, below 60 °C, the copolymers had high degradation temperature and thermal stability comparable to that of (PCL), and increased hydrophilicity as well as faster degradation time than PCL.

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Acknowledgments

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Functionalization of Polysaccharide Gellan Gum with Avidin to Form Modular Hydrogels

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Introduction

We have designed modular hydrogel for 3D cell culture applications based on the polysaccharide gellan gum. Modularity was achieved through avidin-biotin binding, where avidin is covalently coupled to the polymer network, which in turn can be equipped with biotinylated cell cues. The model polymer gellan gum forms ionic hydrogel using bioamines and has previously been investigated for tissue engineering applications.

Carbodiimide coupling is used as functionalization reaction, which is a well-established method using NHS and EDC.[1] This reaction occurs under mild conditions in aqueous conditions, creates no harmful substances or reaction products, and is thus compound useable for tissue engineering.

Experimental methods

A solution of sodium-purified gellan gum (NaGG, 10 mg/mL, 20 mL) was dissolved in HEPES buffer (50 mM, pH 6.5), stirred at 40 °C, and activated with EDC (0.4 M) and NHS (1.0 M) for 15 min and consequently quenched with β-mercaptoethanol (28 µL, final concentration 20 mM). Finally, charge-neutralized chimeric avidin (1 mg/mL in HEPES 50 mM, pH 6.5) was added and stirred for 5 h at 40 °C. The product was dialyzed over 5 days (MWCO 12-14 kDa) and subsequently lyophilized.

The final product has been analyzed using fluorescence titration, electrophoresis, confocal microscopy, compression and rheological testing.

Results

Sodium-purified gellan gum (NaGG) was functionalized with chimeric charge-neutralized avidin (avd), to yield a modular hydrogel (NaGG-avd).[3] EDC activation is most efficient in acidic conditions, while the amine coupling step should be carried out at physiological conditions. The buffer pH at 6.5 was chosen to balance these aspects.

The product was rigorously analyzed for avidin attachment, physico-mechanical properties of the hydrogel, as well as cell response. Based on quenching response of the fluorescence dye when bound to avidin, titration with a biotin dye (b-5-fluorescein) revealed a concentration of 0.1-0.3 nmol/mg NaGG in the polymer. Electrophoresis (urea SDS-PAGE) was employed to confirm the covalent binding between NaGG network and avidin, in comparison to a physical mixture of the components. Indeed, a strong difference between the bands is visible. Gel samples with biotinylated fluorescence dye were prepared to show stability and presence of avidin in the gelated network after washing steps, from the mean brightness of a 30µm thick confocal microscopy z-stack.

Hydrogels are formed from the precursor (NaGG-avd in buffer) using cationic bioamines (spermidine) as cross-linker[2], allowing for the encapsulation of cells in mild and cell culture-relevant conditions. Compression behavior is remarkably similar between NaGG-avd and NaGG hydrogel samples, and mostly dependent on choice and concentration of the crosslinker. Bioamine crosslinkers (spermidine) form softer hydrogels with lower fracture strain and strength, compared to traditional Ca^{2+} crosslinker. Increase in cationic strength, also introduced through cell culture medium, increases stiffness of the hydrogel.[4] Preliminary rheology tests revealed gelation behavior and modulus of cell-laden gels.

Conclusion

We have shown to functionalize gellan gum with avidin using carbodiimide chemistry in a simple and robust one-step reaction. Various analysis techniques have been used and shown the feasibility of this hydrogel platform.[3] The mechanical property range is appropriate for biofabrication techniques and cell encapsulation. This modular hydrogel can also be envisioned to be used as bioink for 3D printing.

Acknowledgments

This work was supported by Business Finland Human Spare Parts project and Academy of Finland Center of Excellence Body-on-Chip project (n:o 312409).

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Nanomanufacturing and functional behavior of disulfide-bearing nanoparticles

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Introduction

Here, we tackled two points in the preparation of nanocarriers for drug delivery. A) The production of bioreactive nanoparticles in a microfluidic-assisted fashion[1], using poly(ε-caprolactone)-b-poly(ethylene glycol) with a thiol-exchangeable pyridyl disulfide group (pds) on the PEG-terminus (PCL-PEGpds)[2]. B) The stability of a disulfide-based bioconjugation in biologically relevant conditions.

Experimental Methods

PCL-PEG-methoxy and PCL-PEGpds were provided by Sigma Aldrich (5.5 kDa PCL, 6 kDa PEG). Thiol-containing peptides with various ionic residues (double positive RR, neutral RD, anionic DG and double negative DD) flanking a cysteine were obtained from Biomatik. Nanoparticles were prepared by mixing polymer in acetone with water in a microfluidics chip (Micromixer, Syrris Ltd.) (organic/water ratio = 0.5) with varying total flow rates (TFR). Particle size was determined by dynamic light scattering (DLS) and asymmetric flow field flow fractionation (AF4). Particles were functionalized with peptides (peptide/pds = 3 mol/mol) at 37 °C in PBS. Pds release kinetics was monitored via UV-Vis, the peptide release via HPLC. Internalization kinetics was studied in HCT116 cells, evaluating the fluorescence of pyrene-containing particles in cell lysates.

Results and Discussion

The average particle size (DLS) is adjustable in the range 80 to 160 nm using the microfluidic TFR. AF4, however, showed 20-60 nm particles to be the main constituent of all suspensions irrespective of the TFR value. Further, TFR did not affect the availability and reactivity of pds groups on PCL-PEGpds particles (figure 1). On the contrary they were very much dependent on thiol pKa, which also affected the release of the peptides upon exchange with glutathione (figure 2a); importantly, even the most rapidly exchangeable disulfides (double negative DD peptide) were stable for several hours, differently from pds, which rapidly exchanged with glutathione. The particle uptake by HCT116 (figure 2b) reflected the stability of the peptide functionalization, with the glutathione presence only affecting the uptake kinetics of pds-containing particles.

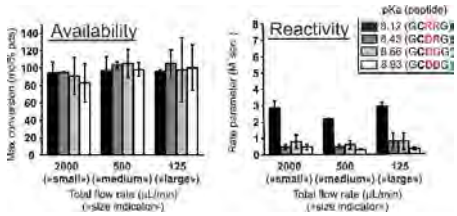

Figure 1. Reaction parameters for peptides with PCL-PEGpds particles formed at different TFR.

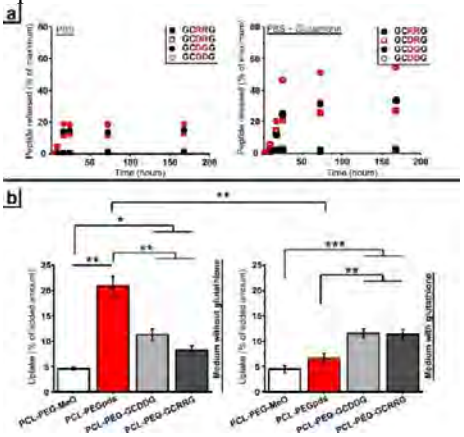

Figure 2. (a) Peptide release from nanoparticles. (b) Uptake by HCT116 cells.

Conclusions

Using PCL-PEGpds nanoparticles, we have demonstrated the stability of pds functionalization in microfluidic-prepared particles.

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Acknowledgments

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Osteodifferentiation on porous poly(trimethylene carbonate)/hydroxyapatite composites

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Introduction

Treating critical-size bone defects requires porous, osteoconductive structures that allow for bone ingrowth (>300 μm pores) and nutrient distribution (<20 μm pores)[1]. Here, porous composite films of photo-crosslinked poly(trimethylene carbonate) (PTMC) and hydroxyapatite (HA) were prepared using temperature-induced phase separation. The effect of varying a small-scale porosity (<20 μm width) on osteodifferentiation of human bone marrow mesenchymal stem cells (hBMSCs) was investigated.

Experimental Methods

Three-armed PTMC with methacrylate termini (10.5 kg/mol) was synthesized as described[2] and HA was provided by Kuros Biosciences BV. Porous films were prepared by mixing PTMC, HA (PTMC/HA = 1.5 (w/w)), Omnirad TPO-L and ethylene carbonate (EC) at 50 °C, followed by solvent casting, cooling to crystallize EC, photo-crosslinking (365 nm, 11 mW/cm^2, 30 min, N_2) and EC extraction. The EC content was varied to adjust porosity of the final films, which was determined gravimetrically. Pore size was investigated by SEM. Mechanical properties were determined in tensile experiments. hBMSCs were seeded on films in osteogenic medium. Cell attachment was determined by DNA quantification. Osteogenic differentiation was followed through alkaline phosphatase (ALP) activity and Alizarin Red staining. Significance was assessed using a two-tailed Welch's t-test (setting $p < 0.1$).

Results and Discussion

Porous composite films with porosities of 27, 52 and 71% were prepared. The average pore width on the film surfaces was similar and ranged from 5 to 10 μm. Stiffness of the films declined with an increasing porosity from 70 MPa at 27% to 3.5 MPa at 71% porosity. Cell attachment on the composite films was initially enhanced with increasing porosity, but was similar at later times (figure 1A). The ALP activity of the cells did not show significant differences (figure 1B). However, in terms of calcium production a significant reduction was observed as the porosity of the films increased (figure 1C), indicating a weakened osteodifferentiation.

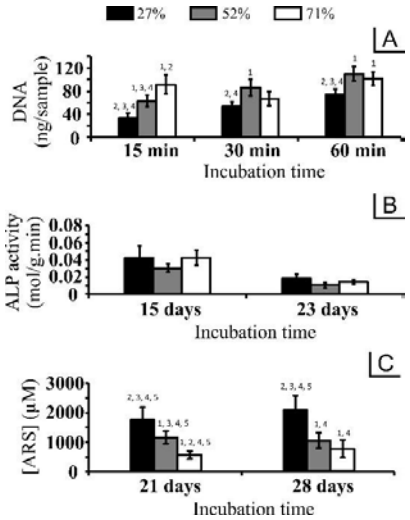

Figure 1. hBMSC attachment (A), ALP activity (B) and calcium production (C) on porous composite films.

Conclusions

We demonstrate that varying small-scale porosity (<20 μm width) in photo-crosslinked composites of PTMC and HA does not have a strong effect on cell attachment, but does affect the osteodifferentiation of hBMSCs.

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Acknowledgments

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Nerve regeneration using composite PTMC/rGO nerve guides: A pilot study

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Introduction

Peripheral nerve injuries are clinical problems that draw much attention in both materials science and biology. Current treatments, such as using autologous nerve grafts to bridge the ends of the damaged nerve, are unsatisfactory. Strategies based on synthetic nerve guidance conduits have also been developed and applied in clinic. Only short gaps could be repaired. Regeneration times were long and functional recovery was poor.

In previous studies, electrically conductive poly(trimethylene carbonate) and reduced graphene oxide (PTMC/rGO) composites were prepared and were shown to have good compatibility with PC 12 neuron cells [1]. Nerve conduits based on PTMC/rGO composites can be expected to promote peripheral nerve regeneration *in vivo*.

Experimental Methods

Three-armed PTMC (19000 g/mol) was synthesized by ring-opening polymerization and functionalized with methacrylic anhydride to yield a crosslinkable compound (PTMC-MA). Porous PTMC and PTMC/rGO composite nerve conduits were made by dip-coating and particle leaching.

PTMC-MA or PTMC and rGO (2wt% relative to PTMC-MA) were dissolved in chloroform. NaF (2 to 50 μm) porogen particles (70vol% relative to the total of the solids) and Irgacure 2959 photo-initiator (2.5wt% relative to PTMC-MA) were added to the solution. A glass mandrel (3mm diameter) was coated with the polymer mixture by dipping it into the solution several times. The polymer coating was then dried and photo-crosslinked by UV. It was subsequently extracted with chloroform/ propylene carbonate mixtures, then the NaF particles were leached out using distilled water and dried. The morphology of obtained porous tubular structures was observed by SEM.

The PTMC and PTMC/rGO nerve conduits were implanted in rabbits for a period of 6 weeks to bridge sciatic nerve defects of 15mm. Histological analyses were performed after methylene blue and fuchsine staining.

Results and Discussion

As shown in Figure 1A and B, 20mm long porous tubular PTMC and PTMC/rGO composite nerve conduits with 3mm inner diameter were obtained. The pore sizes were 2-60 μm; the porosity of the PTMC and the PTMC/rGO composite conduits was 71% and 73%, respectively.

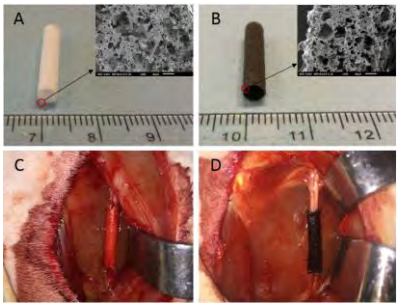

Figure 1*. Macroscopic images and SEM images of PTMC (A) and PTMC/rGO composite (B) conduits. Implantation of PTMC (C) and PTMC/rGO composite (D) conduits bridging rabbit sciatic nerve defects.*

Both conduits were successfully implanted. Figure 2 showed that at 6 weeks axon bundles could be observed for the sciatic nerve itself and for the tissue regenerated at the middle of the graft when using the PTMC/rGO composite. The nerve regenerating within the PTMC conduit did not reach the middle of the graft at 6 weeks.

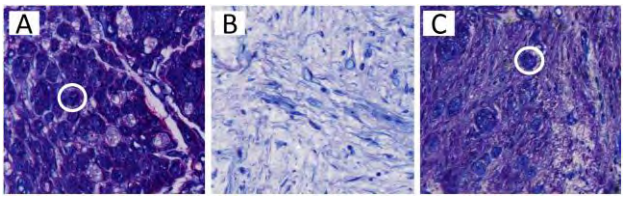

Figure 2. *Histology images of native sciatic nerve (A), PTMC conduit (B), PTMC/rGO composite conduit (C). Axon bundles stained dark blue (in white circles), collagen stained red. Images B and C are of cross-sections at middle of the graft. Magnification: x40.*

Conclusions

Composite PTMC/rGO nerve conduits showed successful regeneration of damaged sciatic peripheral nerves in the rabbit at 6 weeks. Use of comparable nerve conduits based on PTMC did not show nerve regeneration.

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Acknowledgments

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The Particle With the Golden Gun

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Introduction

Gold nanoclusters have received increasing attention for biomedical applications in recent years due to their interesting physical properties and physiological compatibility. Gold nanoclusters in the few nm size range can transduce near infra-red light into heat, which is a property that can be utilized for laser targeted drug delivery.[1] Furthermore, when approaching the sub <2nm size range gold nanoclusters exhibit luminescent properties that can potentially be employed for diagnostic applications.[2] Here, we describe a core-crosslinked micellar system designed to contain gold nanoclusters and thiol derivatized chemotherapeutic doxorubicin (DOX) for laser targeted cancer therapy.

Experimental Methods

Diblock polymers of Polyethylene glycol-Poly(N-isopropylacrylamide -*co*- 2-Hydroxypropyl methacrylamide-Cysteine) abbreviated as PNC and Polyethylene glycol-Poly(N-isopropylacrylamide -*co*- Acrylic acid N-hydroxysuccinimide ester) abbreviated as PNN were synthesized via RAFT polymerization employing PEG_{5000} derivatized with a RAFT chain transfer agent. Core-crosslinked micelles were formed with a 2:1 PNC:PNN mixture in PBS at 50°C via Oxo-ester Mediated Native Chemical Ligation (OMNCL). Gold nanoclusters were then formed within the core-crosslinked micelles at a 3:1 thiol to $HAuCl_4$ ratio by reduction with $NaBH_4$. DOX was modified with iminothiolane, introducing a thiol moiety (DOX-SH), and included during the gold reduction step into the micelles.

MDA-MB-231 cells were cultured in the presence of micelles for 24 hours. Life/dead staining was done using calcein/PI after illumination a small spot in the well for 60 min with a laser of 650 nm at 300 mW.

Results and Discussion

Polymers were successfully synthesized as identified by NMR and show cloud points of around 30°C (see table 1). The empty core-crosslinked micelles were found to have a size of 60 nm and 70 nm when containing gold nanoclusters at 50°C with a PDI of 0.13 and 0.16 respectively, which indicates retained micelle stability after the reducing conditions.

DOX-SH was successfully entrapped within the micelles and can be released by addition of reducing agents including tris(2-carboxyethyl)phosphine, indicating thiol based bond involvement in the DOX entrapment.

MDA-MB-231 cells treated with the particle formulation and laser undergo apoptosis only in the focal point of irradiation (see figure 1). Controls indicate that only the formulation with micelles containing both gold nanoclusters and DOX-SH leads to successful cell killing. An incubation time of 24 hours after irradiation is necessary before staining, affirming DOX is involved in the mechanism of cell death. Synergistic action between the heating effect of the gold nanoclusters and DOX is also expected to play a role in the increased cytotoxicity.

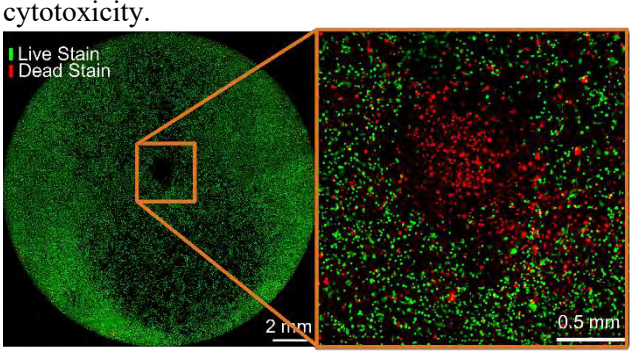

Figure 1. Life-Dead assay of MDA-MB-231 cells treated 24 hours with the gold-DOX loaded PNC-PNN micelles and subsequently irradiated 60 minutes with a 650 nm laser at 300 mW. Cells were stained 24 hours after laser irradiation

Table 1. Polymer Characterization.

	% NIPAM		% (HPMA-Cys or NAS)		M_n (kDa)[a]	M_n (kDa)[b]	PDI[b]	Cloud Point (°C)
	Feed	Obtained	Feed	Obtained				
PNC	93	93	7	7	15.9	16.0	1.5	33
PNN	93	93	7	7	15.3	15.0	1.4	29

[a] Determined by ^1H NMR [b] Determined by GPC

Conclusions

The herein described particle formulation can successfully be employed for triggered localized cytotoxic treatment of cancer cells.

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Microstructures of hydrazone crosslinked hydrogels determined using rheology- and diffusion-based studies

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Introduction

Microstructure influences strongly how suitable hydrogels are as biomaterials, especially when drugs or cells are encapsulated inside the hydrogel. The mechanical and diffusional properties of hydrogels are dictated by the structural parameters, such as mesh size and crosslinking density. Indeed, the mesh size has been used to correlate the diffusivity of molecules inside the hydrogels. The aim of this study was to characterize the microstructures of hydrazone crosslinked hyaluronan (HA), gellan gum (GG) and alginate (AL) -based hydrogels using rheology- and diffusion-based methods.

Experimental Methods

The microstructures of hydrazone crosslinked HA-polyvinyl alcohol (PVA)-, AL-PVA-, GG-HA- and HA-HA(-collagen I)-based hydrogels[1-3] were evaluated using rheology- and diffusion (fluorescence recovery after photobleaching, FRAP)-based methods[4]. The effect of the gel parameters (degree of substitution and molecular weight of gel components, ratio of gel components, and polym. conc. of hydrogel) on the viscoelastic and diffusion properties of hydrogels, and further to their structural parameters (mesh size, crosslinking density and average molecular weight of the polymer chain between neighboring crosslinks) were studied. Statistical data analyses were performed for rheological and FRAP data set using MATLAB (Kruskal-Wallis test and Wilcoxon rank sum test, p-value of <0.05 was considered significant).

Results and Discussion

FRAP-tests showed that the diffusivity decreased when larger dextran sizes (500-2000 kDa) were used. Those molecule sizes were equivalent to the mesh sizes of hydrogels (15 nm to 47 nm) determined by the rheological method. This mesh size range allows the transportation of smaller molecules, peptides and most proteins (Fig. 1.). The rheological results also showed a proportionality between the structural parameters and storage modulus. The structural parameters and FRAP results also supported the findings of our previous cell tests[1-2].

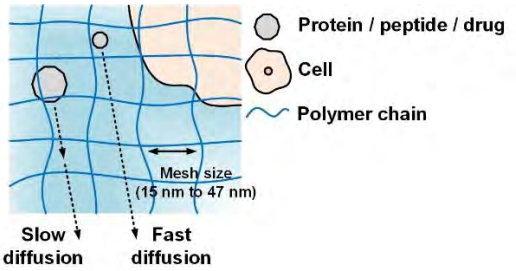

Figure 1. Reasonable mesh size range for the transportation of molecules through the hydrogels.

Conclusions

The microstructures of these hydrogels can be evaluated using rheology- and FRAP-based methods. Hydrazone crosslinking offers an easy way to produce hydrogels at room temperature with variable microstructures as well as variable viscoelastic and diffusion properties suitable for soft tissue engineering applications, by altering the gel parameters.

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Acknowledgments

This work was supported by Business Finland Human Spare Parts project and Academy of Finland Center of Excellence Body-on-Chip project (n:o 312409). The authors acknowledge Tampere Imaging Facility (TIF) for their service.

Wood-derived nanofibrillar cellulose as a cell scaffold for wound treatment

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Introduction

Due the ineffective treatment methods, chronic wounds represent a major clinical challenge for healthcare professionals. Stem cell transplantations have shown great promise to promote wound healing. However, a hostile environment of the injured tissue has shown considerably to limit the survival rate of the transplanted stem cells[1]. Therefore, stem cell survival and retention are subjects to be improved towards successful cell therapy. One potential approach for that purpose is the use of biomaterials as a cell scaffold. In this project, interactions between wood-derived nanofibrillar cellulose (NFC) dressing previously used in clinic for skin graft donor site treatment[2] and multipotent human adipose-derived mesenchymal stem/stromal cells (hASCs) are studied in order to develop cell transplantation method for wound care. Our hypothesis is that the NFC offers an efficient culture platform for hASCs and support the cell survival and function.

Experimental Methods

During the project, characteristics of hASC cultured on NFC wound dressing (UPM Kymmene-Corporation, Finland) were evaluated using cell viability assays, scanning electron microscopy (SEM), immunocytochemistry (ICC) and quantitative PCR. Student's t-test was used to determine statistical significance.

Results and Discussion

hASCs cultured on NFC wound dressing showed high cell viability during two-week culturing without any cell adhesion coatings. In addition, NFC dressing did not induce any remarkable cytotoxicity or alter the morphology, cytoskeletal structure or gene expression of hASCs. Therefore, it can be stated that NFC dressing maintains the function and undifferentiated state of hASCs during the culturing, and thus, serve as a promising material to function as a cell scaffold.

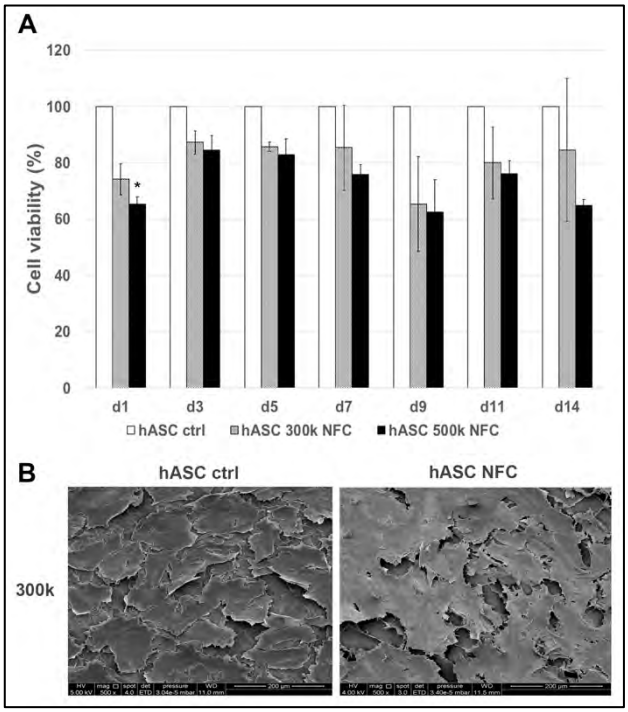

Figure 1. Culturing hASCs on top of NFC dressing with 300 000 cell/cm^2 (300k) and 500k cell density. **A)** High cell viabilities were observed especially with 300k cell density with no statistically significant difference (*p<0,05**)** compared with the control cells during the two-week culturing. All values are mean (±SEM, n=3). **B)** SEM micrographs from hASCs cultured with 300k cell density on top of NFC dressing showing similar morphology compared with the control cells.

Conclusions

NFC dressing is an efficient culture platform for hASCs and offers a potential cell therapy method for wound healing in the future.

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Fabrication of Fiber Actuators Suitable as Muscles in Robotics

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Introduction

Shape-memory polymer based soft actuator materials, which are capable of defined, programmable thermoreversible movements have emerged as promising candidate materials for applications in soft robotics [1]. Although, such actuator materials can be fabricated from commodity thermoplastics [2], the required multi-step modification procedures, including covalent crosslinking and mechanical programming represent hurdles for their application in (soft) robotic systems, which we would benefit from an electrically triggerable actuator system. To overcome this limitation, SMPA enabling the electrical control of the shape-shifting, which can be realized in simple and up-scalable fabrication schemes are required. Here, we present a fabrication approach for realizing electro-conductive poly[ethylene-*co*-(vinyl acetate)] (PEVA) based fiber actuators, which show electrically driven reversible movements neither requiring chemical modification nor programming.

Experimental Methods

PEVA with a vinyl acetate content of 28 wt% (Elvax® 260A, DuPont) and carbon black (Super P conductive, 99+%, Alfa Aesar) were used to prepare electro-conductive actuator fibers by extrusion and subsequent coating. The thermal and mechanical properties of the fibers were studied by differential scanning calorimetry (DSC) and tensile tests. Scanning electron microscopy (SEM) experiments were untilized to inspect the surface coating of the actuators. The Joule heating behavior was examined by exposing the fibers to voltages between 30 and 70 V, while the change in temperature of the fibers was monitored with an IR camera (Jenoptik AG, Jena, Germany). The actuation performance of the obtained fibers was quantified by cyclic thermomechanical and cyclic electromechanical tests.

Results and Discussion

Electro-conductive actuator fibers with a diameter of 400±100 µm were fabricated from PEVA28 by extrusion and subsequent coating with a mixture of poly(ε-caprolactone) and carbon black (PCL/CB). SEM investigations revealed a homogeneous surface coating of the fibers with a PCL/CB layer having a thickness of 6±1 µm. DSC experiments confirmed a broad melting transition for the PEVA fibers ranging from 40 to 80 °C with a melting peak at $T_m \approx 67\pm2$ °C and a crystallization peak at $T_c \approx 44\pm2$ °C. Tensile tests at ambient temperature revealed a Young's modulus of $E \approx 41\pm5$ MPa and a high elongation at break ($\varepsilon_b \approx 600\pm30$) for the fibrous actuators. By applying a voltage of 30 V for approximately 30 s, the temperature of the actuator fiber could be increased to 60 °C as measured by IR-camera, while after switching off the electric current the actuator temperature decreased to ambient temperature within a similar time. The repetitive Joule heating to 60 °C and cooling to ambient temperature caused a free-standing reversible change in the length of the fiber actuator of $\Delta L = 2.5\pm0.5$mm. The observed electromechanical performance was similar to the thermomechanical of the fiber actuator.

Conclusions

The current study demonstrated the fabricated electrically controlled actuation of CB coated PEVA fibers can be achieved, without programming of the fibrous SMPA. In perspective, the alteration of the mechanical performance with degradation can be studied.

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Acknowledgments

Bundesministerium für Bildung und Forschung
Helmholtz-Graduate School Macromolecular Bioscience

Tough and Biocompatible Hybrid Networks Prepared from Methacrylated Poly(Trimethylene Carbonate) (PTMC) and Methacrylated Gelatin

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Introduction

Preparing functional tissue engineered blood vessel is a meaningful approach to solve the limitations of autologous blood vessel transplantation. Poly(trimethylene carbonate) (PTMC) has shown to be a promising material with which to prepare blood vessel scaffolds[1]. Networks made from PTMC are flexible, elastic and have shown to be compatible with different cell types and degraded in vivo by a surface erosion process[2]. In this work, hybrid networks made from functionalized PTMC and -gelatin (Gel) were explored. We hypothesized that by incorporating a natural polymer into the synthetic polymer network, cellular adhesion may be enhanced.

Experimental Methods

Methacrylated PTMC and -gelatin (PTMC-dMA and GelMA) were separately dissolved in DMSO/formic acid (90:10 v/v) at a concentration of 30 wt.%. Irgacure 2959 (1 wt.% relative to the macromer) was added as a photo initiator. Three different mixed macromer networks were prepared by mixing the PTMC-dMA and GelMA solutions at 75:25, 50:50 and 25:75 ratios (v/v). Next, the solutions were photo-crosslinked by UV irradiation for 60 min at 365 nm. Then, the sol fraction was extracted in DMSO/ethanol (90:10 v/v) after which the DMSO was gradually exchanged for ethanol. Finally, the network was dried under vacuum at room temperature. The mechanical properties were assessed by tensile testing. Smooth muscle cell (SMC) attachment to the surface of the networks was determined by a Presto blue assay after culturing for 1, 4 and 7 days.

Results and Discussion

Compared to single polymer networks, all hybrid networks were opaque (Figure 1 (a)). This is due to phase separation of the PTMC and gelatin. The gelatin incorporated in the networks provided enhanced cell attachment as shown in Figure 1 (b). Tensile testing of the 75:25 (v/v) PTMC-dMA-GelMA network revealed an elongation at break and toughness of 410 % and 316 N/mm^2, respectively (Figure 1(c)). For the 50:50 and 25:75 PTMC-gelatin networks these values were significantly lower.

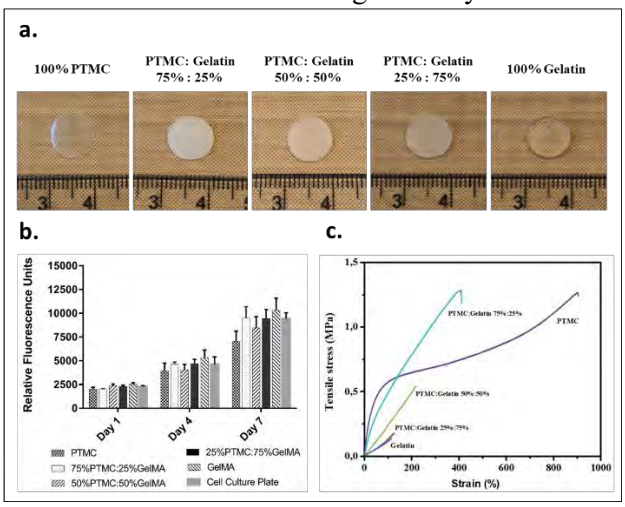

Figure 1. (a) Single- and hybrid PTMC-dMA and GelMA networks in the wet state. (b) Viability of SMC on PTMC-dMA, GelMA and PTMC-dMA-GelMA hybrid networks after 1, 4 and 7 days of culturing. (c) Stress-strain curves of networks in the wet state.

Conclusions

It is concluded that networks composed of 75% PTMC-dMA and 25% GelMA showed very good mechanical and biological properties, and could be considered promising materials for use in preparing tissue engineered blood vessels.

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Acknowledgments

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Shape Recovery of Polymeric Nanocomposite Foams by Direct and Inductive Heating

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Introduction

Polymeric nanocomposites can be stimulated remotely by inductive heating of magnetic nanoparticles.[1,2] So far this stimuli has been reported on non-porous materials. Foams prepared by thermally-induced phase separation (TIPS) have shown shape-recovery by direct heating.[3] It is being hypothesized that highly porous nanocomposite foams can be prepared by TIPS into which magnetic particles can be incorporated and evenly distributed which enable a stimulus by direct heating and alternatively by remotely controlled inductive heating in a magnetic field.

Experimental Methods

Foams have been prepared from aliphatic polyetherurethane (Tecoflex EG72D (TFX); Noveon Thermedics, Wilmington, MA, USA) to which magnetic nanoparticles[1,2] (2.5, 5, 10 wt%) were added. The solvent was removed by freeze-drying.
The foam morphology was investigated by scanning electron microscopy (LEO 1550 VP electron microscope (Zeiss, Jena, Germany)) and x-ray microcomputer tomography (µCT; Procon X-ray GmbH, Garbsen, Germany). The foams were compressed at T_{prog} = 80 °C to 50% of their original height, followed by a fixation at approximately T_{fix} = 0 °C. The shape recovery was investigated in a heat chamber, dynamic thermomechanical analysis (DMTA; Zwick Z1.0, Zwick, Ulm, Germany) or alternatively in a magnetic field having a frequency of 258 kHz (TIG 5/ 300; Huettinger Electronic, Freiburg, Germany).

Results and Discussion

Open-porous, nanocomposite foams were prepared, which showed a bimodal size-distribution with large pores with sizes above 100 µm and smaller pores (approx. 10 µm) located in the cell walls. The nanoparticles were evenly distributed according to the eye. The shape recoveries of the compressed foams were induced by three different methods: heat chamber, DMTA (both T = 80 °C) or heating inductively in a magnetic field. A shape recovery R_r of upto 76 ± 4% was reached in the heat chamber, while the DMTA revealed recoveries up to 68±4%. In case of inductive heating of the compressed foam in the magnetic field, the highest recovery rate found was R_r = 65±4%. The lower recovery rate may be explained by the heat transfer to the environment, which depends on the surface to volume ratio,[1] and the decrease of particle density during the shape recovery process.

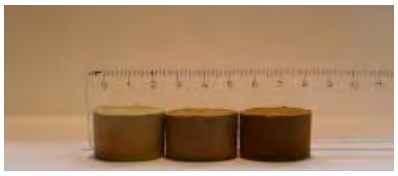

Figure. The photo shows the foams after removal of the top layer in their original state. Notice the increase in color as particle content increases from 2.5 wt% to 10 wt% (from left to right).

Conclusions

Nanocomposite foams were prepared by TIPS. Shape recovery in the heat chamber was highest of all three methods. Shape recovery in the DMTA was lower than in the heat chamber presumably because of the back-pressure needed for the DMTA to determine the height of the specimen during analysis. A shape recovery was also found by inductive heating in a magnetic field. However the recovery rate was lowest of all three methods, which may be explained by the heat transfer to the environment hindering the foam from reaching the necessary temperature.

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Acknowledgments

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Towards a prediction of polymer degradation with quantitative analysis of Langmuir monolayer degradation experiments

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Introduction

Improving the state of the art of treatmens in regenerative medicine demands for materials that are capable of accomplishing multiple tasks, e.g. providing mechanical support while releasing a drug and then vanishing from the body. Such highly multifunctional materials require tailored molecular architectures. Predicting how the molecular architecture affects the degradation behavior and thereby the temporal evolution of all material parameters is essential for designing these materials. Due to the complex interplay of diffusional processes, chemical reactions and the material's microstructure, a prediction neccessitates numerical simulations. The accuracy of computational models depends on the validity of the input parameters. The Langmuir monolayer degradation technique can be used to determine the kinetic parameters of the chemical reactions leading to polymer degradation in aqueous environments in experiments taking less than a day[1]. Such a quantitative analysis requires the application of kinetic models. Here, kinetic models for the degradation of arcitectured chains are presented and applied to experimental results.

Experimental Methods

Polymers were dissolved in chloroform and applied to the air-water interface on a Langmuir trough. The monolayer was compressed to a preset surface pressure and degradation was induced by altering the pH or inserting enzymes. The surface pressure was kept constant and the dissolution of small water soluble fragments was compensated by a gradual reduction of the monolayer area.

Results and Discussion

The hydrolytic degradation of multiblock copolymers, homopolymers and polymers with non-degradable moieties at the chain-ends is adequately described by the models. The ε-caprolactone based polymers degraded via random fragmentation. The bond scission rate constants were on the order of 10^{-5} s^{-1}. In multiblock copolymers, molecular weight and block length had little effect on the degradation rate. The presence of non-degradable crystallites in the layer reduced the degradation rate of non-crystalline chains as well. The evolution of the molecular weight can be calculated from the models as examplified for homopolymers where it decreased logarithmically. Future research will focus on other degradation mechanisms than hydrolysis leading to break down of materials considered as non-degradable.

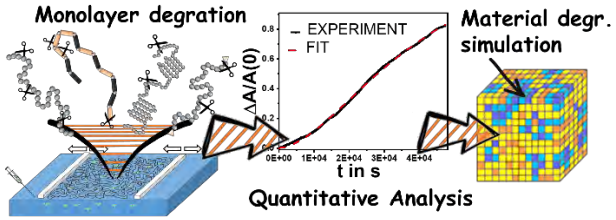

Figure 1: The degradation of architectured macromolecules was investigated with Langmuir Monolayers. Kinetic parameters were extracted via quantitative analysis and will be used for numerical simulations of material degradation in the future

Conclusions

With the kinetic models presented here, the degradation kinetics of polymers with complex architectures can be analyzed. The results will be used for numerical and analytical approaches to predict the degradation kinetics of polymer materials.

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Co-transfection Strategies for In Vitro Transcribed mRNA

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Introduction

The efficiency of non-viral gene delivery systems made such progress in the last three decades, that they became a viable alternative to e.g. retro- or adenoviral gene transfer[1]. In many experimental settings, addressing either fundamental scientific questions or therapeutic applications, co-transfection of two genes at the same time into individual cells is of utmost importance[2]. Examples are the expression of heterodimeric proteins or the co-introduction of an imaging marker. The question was addressed, whether often overlooked technical parameters like order-of-addition in the experimental design of co-delivery studies might play a crucial role in the utility of the delivery protocol. We show for the co-transfection of mRNA that the preparation of the gene transfer mixtures, in the given example liposome-based, has a remarkable impact on the distribution of cells co-expressing the genes of interest.

Experimental Methods

In this study mRNA was chosen as an examplary nucleic acid for co-transfection of cells, as the readout is not confounded by differences in nuclear uptake. mRNA was synthesized via in vitro transcription method (IVT). Briefly, plasmid vectors based on pRNA2-(A)128 and pRNA2-(A)128-mCherry coding Green Florescent Protein (GFP) and mCherry, respectively, were linearized and used as DNA template for mRNA synthesis (TranscriptAid T7 High Yield Transcription Kit, K0441, Thermo Scientific) based on the manufacturer's instruction.

In the next step, the co-transfection mixture (i.e. mRNA and Lipofectamin Messenger Max (LipoMM; Thermo Scientific) as a transfection reagent) was prepared either by mixing GFP and mCherry mRNA before mixing with LipoMM ("Premixed" method), or by adding the respective mRNA/LipoMM complexes separately to the same well ("Separated" method). Subsequently, an established human cells line was transfected under either of these two conditions. Transfection efficiency was evaluated 24 h after transfection via fluorescent microscopy and flow cytometry (FACS).

Results and Discussion

The relevance of the co-transfection mixture preparation method was investigated using the two fluorescent protein markers, GFP and mCherry. Results clearly demonstrate that there is a substantial difference in distribution of cells co-expressing both reporter proteins, and cells which express only one of them for the two different co-transfection protocols.

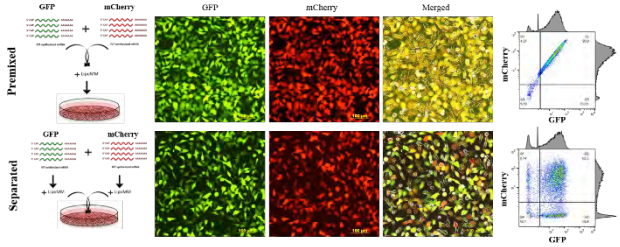

Figure 1. Comparison of two different approaches to prepare co-transfection mixtures and its effect on subsequent transfection efficiency.

FACS results show that 90±3% of cells were expressing both GFP and mCherry in case of "premixed" method, whereas only 62±8% of "separated" transfected cells were double positive (n=3).

Conclusions

The impact of nucleic acid mixing step during co-transfection study has been elucidated and proved to be an important technical parameter for non-viral gene delivery. This has to be taken into consideration when designing co-transfection experiments for clinically relevant genes as well as for mechanistic studies.

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Polymer brush-based vectors for oligonucleotide delivery in 3D hydrogels

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Introduction

RNA delivering scaffolds are emerging and attractive systems to modulate cell phenotype for tissue engineering applications. Successful application of these scaffolds requires to address several technological challenges, including the protection of the RNA from nuclease degradation, prolonged RNA retention and sustained release. We have designed polymer brush-based nanoparticles for prolonged and stable RNA retention for tissue regeneration. These vectors were incorporated within cell degradable fibrin hydrogels, so that they can be accessed as cells spread, proliferate and remodel the hydrogel. The interaction of the polymer brush-RNA complexes with human dermal fibroblasts was studied using 3D cellular uptake and transfection assays.

Experimental Methods

Silica nanoparticle cores (300 nm) were decorated with polymer brushes via the surface grafting of the cationic poly(dimethylaminoethylmethacrylate) (pDMAEMA) using atom transfer radical polymerization (ATRP). The polymer brush grafted silica nanoparticles depicted self-assembly in phosphate buffer saline and were complexed with fluorescently labelled siRNA (FAM siRNA) and incorporated in to fibrin gels to study their release profile and cellular uptake. Vimentin siRNAs were used for assessing transfection efficiencies of the polymer brush-based vectors. Transfection efficiency was quantified by immunofluorescence assay of vimentin protein. The release and delivery of miRNA was then studied, focusing on the regulation of matrix remodeling.

Results and Discussion

Polymer brushes were found to display high capacity to stably bind oligonucleotides, owing to their extreme grafting density (0.5 chains/nm^2). This translated in an improved capacity to retain RNA within hydrogels, in comparison to conventional vectors, and their ability to deliver these oligonucleotides over sustained periods of time. In turn, this resulted in significant transfection efficiencies over 1 week, and results indicate the potential of such strategy to modulate matrix remodeling in tissue engineering platforms.

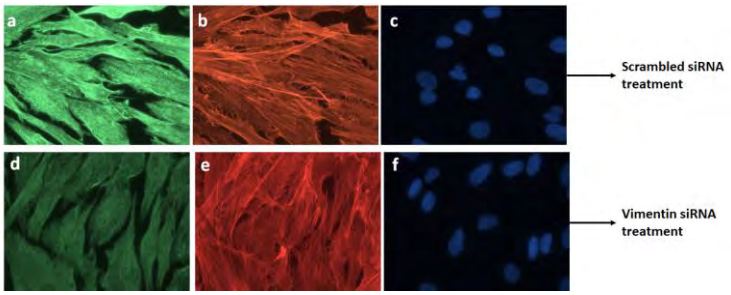

Figure 1. Immunofluorescence assay of fibroblasts cells for vimentin protein after treatment with scrambled siRNA (a-c) and vimentin siRNA (d-e) complexes with polymer brushes. Green channel – vimentin protein, Red channel - Actin filament and Blue channel – Nuclear staining

Conclusions

The results demonstrate the potential of polymer brush vectors for the delivery of oligonucleotides in 3D soft matrices, for tissue engineering applications.

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Intracardiac Echocardiography in transcatheter tricuspid-repair with the MitraClip device: Insights from a 3-D printed heart model

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Introduction:

Transoesophageal echocardiography (TOE) as a guiding tool for transcatheter tricuspid repair may not offer sufficient image quality in a significant proportion of patients [1, 2]. Therefore, alternative guiding tools need to be established.

Experimental Methods:

This observational single center experience reports on the performance of intracardiac echocardiography (ICE) as a guiding tool in catheter based tricuspid repair with the MitraClip device in 6 patients where TOE image quality was poor. The appropriate angulations of the ICE catheter with respect to each commissure of the tricuspid valve were established in a 3D printed heart model based on CT Data of one of the patients.

Results and Discussion:

The procedure was successfully completed under ICE guidance in 4 of the 6 patients. In 2 patients ICE image quality was too poor to support safe guidance, resulting in abortion of the procedure in one patient. Transthoracic echocardiography was successfully used in another patient. The steering maneuvers of the ICE catheter found in the 3-D Model were well applicable in all of the patients included in this case series.

Although we have not observed any ICE related complications in our cohort, it is important to consider that ICE is still an invasive and therefore potentially harmful imaging technique. Furthermore, there are currently no methods available to predict the intraprocedural feasibility of ICE in these patients.

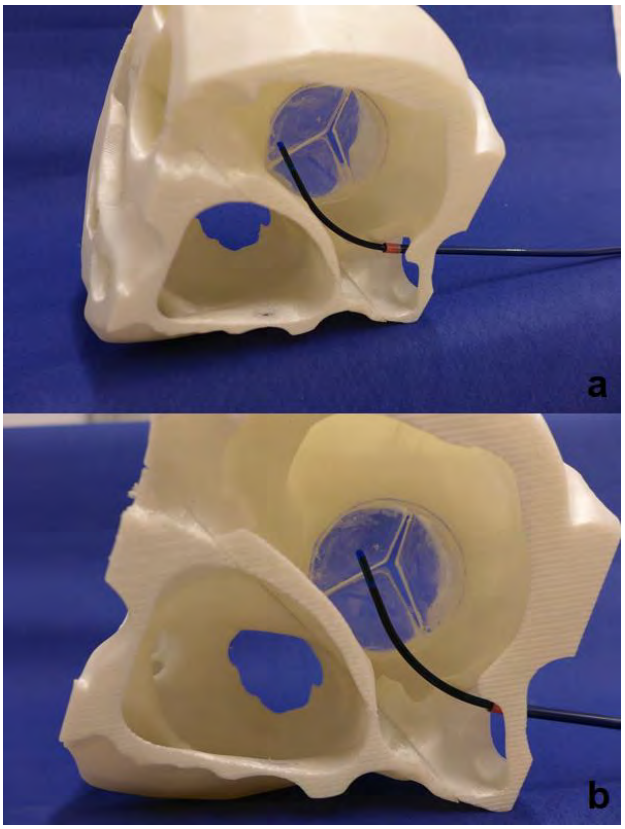

Figure 1. 3-D printed heart model with a right lateral surgical view. Alignment of the anteroseptal commissure. The ICE probe is advanced in the superior portion of the right atrium and slightly deflected anteriorly and lateraly (a). Clockwise/counterclockwise rotation or pulling/advancing the probe will allow to visualize a more central or septal plane of the commissure (a).

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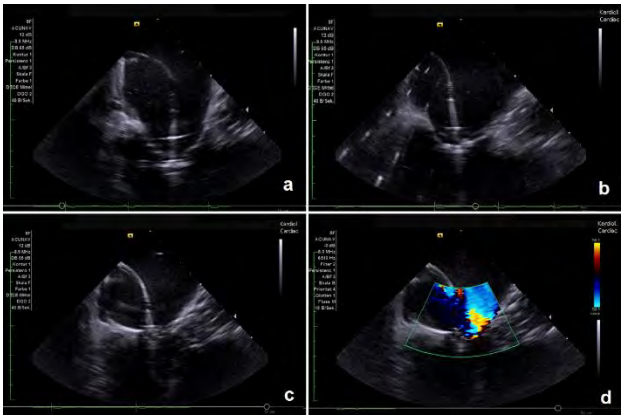

Figure 2. Intraprocedural ICE images of the anteroseptal commissure. The anterior and the septal leaflets in relation to the MitraClip device as well as a previously implanted TAVI prosthesis are clearly visible. Both leaflets resting on the clip arms (a). Lowerig the grippers with sufficient leaflet material inside the clip (b). Sinching of the annulus is clearly visible after closing the clip (c). Color doppler image before the clip is released (d).

Conclusions:

ICE guidance may offer a useful option to support transcatheter tricuspid repair with the MitraClip device in patients where TOE image quality is poor. The mentioned angulations of the ICE probe, which were derived from a 3D printed heart model may work in most patients but significant divergations in different anatomies should be considered. Larger studies are necessary to confirm our experience.

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Dual Material Stereolithography for the Fabrication of Medical Devices

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Introduction

Stereolithography is being widely employed in the fabrication of biocompatible medical devices[1] due to its excellent achievable resolution of up to tens of micrometers. However, one of the main drawbacks of this photo-curing 3D printing method is its limitation in material choice and the difficulty of combining two distinct materials within one printed structure[2].

In the present project, a dual material system capable of polymerizing a compliant polyethylene glycol (PEG) hydrogel and a stiffer epoxy-based material[3] is applied to stereolithography. By choice of irradiation wavelength either the radically initiated PEG-diacrylate or the cationically initiated epoxy compound polymerizes in the designated cross-section area, enabling the directed layer-by-layer fabrication of a dual-material specimen.

Challenges such as chemical characterization of the achieved structures and the slow 3D printing process are addressed in this project.

Experimental Methods

The previously established material system[3] consists of poly(ethylene glycol) diacrylate 700 g mol^{-1} (PEGDA) with camphorquinone (CQ) as a radical photoinitiator irradiated at 455 nm and 3,4-epoxycyclohexanecarboxylate (EEC) with triaryl-sulfoniumantimonate salts as cationic photoinitiator at 365 nm.

An in-house digital light processing (DLP) stereolithography apparatus was employed to print the structures. Material composition was analyzed in IR and Raman spectroscopy and fluorescent labelling of a monomer with subsequent confocal microscopy analysis enabled assessment of printing resolution.

Furthermore, co-initiators to CQ such as ethyl 4-dimethylamino benzoate (EDMAB) and piperonyl alcohol were investigated to accelerate the radical reaction.

Results and Discussion

Dual material structures were successfully printed and it could be shown that when illuminating at 455 nm, exclusively a PEG hydrogel is formed. Upon illumination at 365 nm, on the other hand, the epoxy network is cured in addition to PEGDA.

Accuracy of the printing process could be demonstrated in confocal microscopy and application of different co-initiators reduced necessary irradiation time of the radical polymerization without impeding curing of the cationic system, effectively accelerating the printing process.

As next steps, absence of leachable substances and cytocompatibility of printed structures will be investigated to ensure suitability for applications in the medical field.

Conclusions

The presently developed dual-material stereolithography system could be very promising for potential applications in medicine. High spatial resolution combined with flexibility of working with two vastly different materials can be applied to a variety of medical devices. One example currently pursued is the fabrication of actuators for *in vitro* cardiac tissue maturation for drug safety screening purposes.

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Acknowledgments

None

Oxygen releasing and antioxidant polymeric wound dressing for diabetic wound healing

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Introduction

Diabetic foot ulcers is on the leading causes of non-traumatic amputations in the world. Although wound healing management is an established field, chronic wounds such as diabetic ulcers are still a challenge. Impaired vascularization with lack of oxygen and elevated oxidative stress are some of the key hallmarks of diabetic ulcers. Strategies to overcome these limitation by functionalized polymeric scaffolds or dressing with oxygen releasing and antioxidant properties will find a promising therapy for treatment of these non-healing wounds.

Experimental Methods

We synthesised antioxidant polyurethane and fabricated oxygen releasing antioxidant cryogels (PUAO-CPO) by incorporating of calcium peroxide.1 Scaffolds were synthesised at cryo conditions (-20 C), characterised and studied for oxygen release and antioxidant potential. The effect of oxygen release and antioxidant potential on cell survival under hypoxia and oxidative stress was studied. Finally we demonstrated the effect of these scaffolds in treatment of diabetic wound ulcers in rats.

Results and Discussion

Oxygen releasing antioxidant scaffolds were highly antioxidant nature and released oxygen over a period of 10 days. These scaffolds attenuated oxidative stress induced cell death and alleviated cell survival under hypoxia conditions. In a diabetic animal model, our scaffolds in the form of dressing enhanced wound closure with respect to control groups. By adding biological entities in the form of ADSC derived exosomes further enhanced the rate of wound closure. (Fig 1) Histological analysis showed increased granulation tissue formation and epithelial thickness in oxygen antioxidant scaffolds as compared to control diabetic group, which was further improved by incorporation of exosomes. We observed increased collagen I deposition which is prominent in normal skin tissue, in oxygen and exosome treated groups as compared to control. Further the scaffolds were able to attenuate oxidative stress in the treated wounds which will lead to reduced inflammation and faster healing. Exosomes with oxygen induced neo-vascularization further helping in wound regeneration.. Diabetic wounds are often infected with S aureus and P aeruginosa. So we studied the regenerative effect of the fabricated dressing on infected diabetic wounds. In case of infected diabetic wounds, we observed similar effect however the time of wound closure increased upto 21 days.

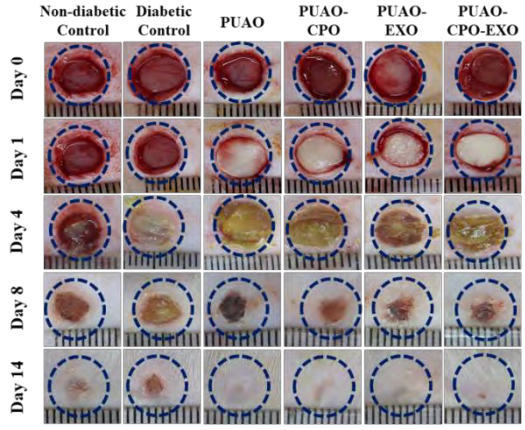

Figure 1: Digital images showing wound closure by application of oxygen releasing antioxidant dressing with and without exosomes

Conclusions

This study demonstrated the effect of an antioxidant oxygen releasing wound dressing for diabetic wound healing. This product will have promising application for treatment of diabetic foot ulcers and will also pave ways to better understand and develop efficient therapies for diabetic foot ulcers.

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Acknowledgments

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Advanced Polymeric Nerve Guidance Channels for Peripheral Nerve Regeneration

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Introduction

Treatment of the peripheral nerve injuries is one of the most complicated problems in the area of reconstructive surgeries. An alternative to gold standard autografts is the development of polymeric nerve guidance channels (NGCs) mimicking the nerve architecture. In the present work, biomimetic advanced polymeric NGCs were designed and developed providing multiple cues in a single structure for enhanced peripheral nerve regeneration.

Experimental Methods

Hollow nerve conduits were fabricated using polyurethane and polycaprolactone (PCL) polymers by using electrospinning and 3D printing. Topographical features in the lumen were fabricated by synthesizing aligned chitosan-gelatin cryogels. NGCs were characterised for mechanical properties and morphological features. In-vitro cell material interaction studies were carried out with Neuro2a cells and primary dorsal root ganglion. In-vivo regenerative potential was analysed in a critical size rat sciatic nerve injury model for 16 weeks. Regeneration was analysed by electrophysiological, morphological and histological studies.

Results and Discussion

Developed NGCs have morphological features similar to that of physiological nerve. We observed that outer conduit wall prevented scar tissue infiltration and inner aligned lumen allowed cellular proliferation & Schwan cell alignment, which are critical for regaining the functionality of damaged nerve. In-vivo regeneration study in a critical size defect demonstrated that electrophysiological parameters of NGCs with nerve growth factor implanted groups were similar to that of autografts. Histological & immunological studies showed presence of healthy regenerated nerve, expressing S-100 (Schwann cells) and NF-200 (Neurons). Also, gastrocnemius muscle recovery was obtained in NGCs implanted group similar to that of autografts. Overall our developed NGCs showed functionality and regeneration equivalent to that of autografts and can be a promising alternative to the autografts.

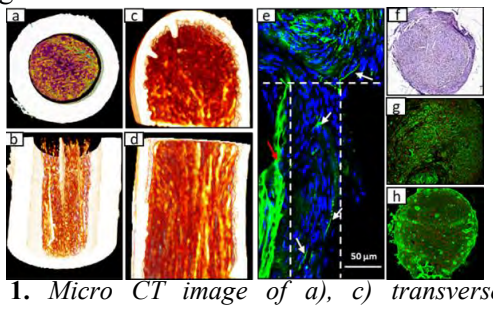

Fig. 1. *Micro CT image of a), c) transverse, b), d) longitudinal section of PCL & polyurethane NGCs; e) Immunofluorescence image of DRG culture showing NF-200 f), Histology & Immunofluorescence image representing g), S-100 & h), NF-200 marker in regenerated nerve.*

Conclusions

The study determines the significance of developed polymeric NGCs providing topographical and biochemical cues allowing cellular adhesion and guided directional growth of regenerating axons. The developed product is a kind of personalised medicinal approach for nerve regeneration.

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Acknowledgments

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Mechanical property of different multiblock copolymers influences endothelial cell behaviors

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Introduction

Endothelial cell behaviors are remarkably influenced by the physical and biochemical signals from their microenvironments [1, 2]. The appropriate mechanical property of the substrate could promote and accelerate angiogenesis process of endothelial cells.

Poly (ether ester urethane) (PEEU) multi-block copolymers, composed of oligo (p-dioxanone) (PDDO) diols and poly (ε-caprolactone) (PCL) diols) segment, are suitable materials for biomedical application [3]. Here, we hypothesized PEEU fiber mesh with different elasticities might influence the proangiogenic behaviors of endothelial cell.

Experimental Methods

PEEU40, PEEU50, PEEU60 and PEEU70 fiber mesh were prepared via electrospinning technique differing in the weight ratio of the two segments. The morphology of fiber mesh was characterized via scanning electron microscopy (SEM) and the diameter of fibers was quantified using Image J software. Young's modulus were measured by tensile test. Cell proliferation, migration, and tube formation of human umbilical vein endothelial cells (HUVECs) was performed at different time points post seeding. Statistical analysis was performed using two tailed independent-samples *t*-test and a significance level (Sig.) < 0.05 was considered to be statistically significant.

Results and Discussion

PEEU fiber meshes with different elasticities were fabricated (As depicted in Fig. 1a). There was no significant difference in fiber diameter. After initial cell seeding, HUVECs proliferated faster on stiffer fiber meshes (PEEU70). There was a 31.9% increase of collective cell migration velocity for PEEU70 compared to PEEU40. The angiogenesis assays revealed much higher numbers of microvascular-like network being formed from HUVEC harvested from PEEU 70 than from PEEU40 (Fig. 1b).

a)

	E-modulus (MPa)	Failure stress (MPa)	Elongation at break (%)	Mesh Thickness (um)
PEEU40	2.64 ± 0.79	1.43 ± 0.40	318 ± 111	39.3 ± 2.7
PEEU50	3.15 ± 0.85	1.14 ± 0.16	154 ± 17	87.3 ± 10.0
PEEU60	3.99 ± 0.89	1.58 ± 0.07	271 ± 11	77.8 ± 3.9
PEEU70	4.54 ± 0.76	2.81 ± 0.17	408 ± 50	53.8 ± 3.9

b)

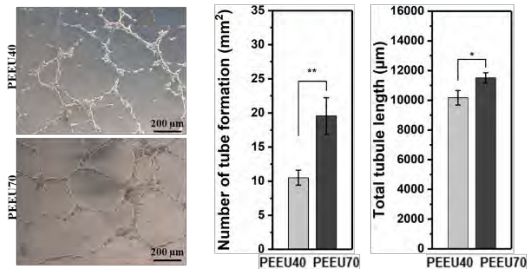

Figure 1. a) Mechanical property of PEEU fiber mesh. b) Tube formation of HUVECs on different fiber PEEU mesh.

Conclusions

Endothelial cells are able to sense and respond to the mechanical property from PEEU fiber mesh. The PEEU fiber mesh with a suitable mechanical property could improve angiogenesis potential of endothelial cells.

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Novel Polymeric Nanocomposite Material for Bioresorbable Stent Application

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Introduction

Bioresorbable stent (BRS) was introduced as the fourth generation of interventional cardiology for treating coronary artery disease. The 'disappearance act' of the device is expected to promote positive remodeling and allowing future reintervention. The current generation of polymeric BRS made of poly-L-lactic acid (PLLA) have significantly thicker struts to compensate for its inherently weaker mechanical properties, and are radiolucent, which makes assessment of scaffold delivery challenging[1]. Hence, it is worthwhile to develop and investigate on a novel material (based on PLLA) with improved mechanical properties (which translates to reduced strut thickness) and radiopacity for BRS application.

Experimental Methods

Functionalized nanofillers ($BaSO_4$, Ta_2O_5 and hydroxyapatite) were melt blended with PLLA (intrinsic viscosity: 8.28) (Purasorb PL, Corbion, Netherlands) with varying weight % via the Xplore Micro 5cc Twin Screw compounder (Geleen, Netherlands) to draw fibres (diameter of 0.2mm & 2mm)The fibres (n=10) were then tested for their tensile properties through the mechanical tester (Model 42 MTS Criterion$_{TM,}$ MN, USA) and imaged for their dispersion and radiopacity under the transmission electron microscopy (Libra 120 plus, Carl Zeiss, Germany) and X-ray machine (Philips Clarity FD20, USA) with an aluminium stepwedge. ImageJ was utilised for all image analysis. Numerical data were analyzed using standard analysis of variance (ANOVA) technique and statistical significance was considered at $p< 0.05$.

Results and Discussion

Loading of nanofillers until a critical wt.% enhances the tensile modulus of PLLA (Figure 1) as it is likely to help impart rigidity to the nanocomposite system. The decrease of tensile modulus could be attributed to the agglomeration of nanofillers as seen in Figure 2[1].

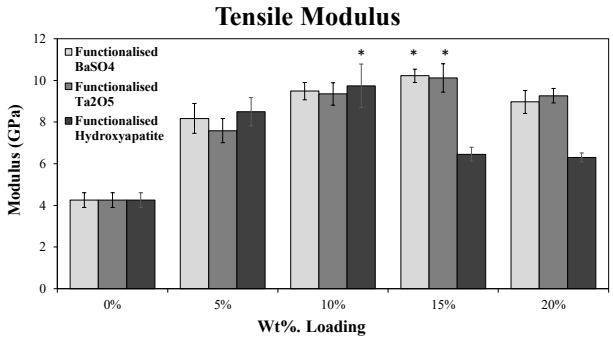

Figure 1. Tensile modulus of increasing loading of functionalized nanofillers in PLLA.

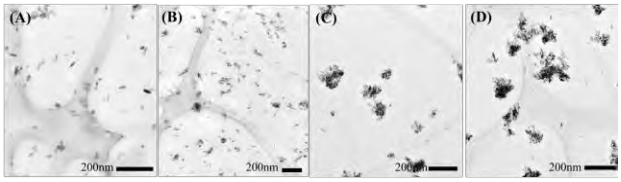

Figure 2. TEM images at different loading of nanofillers, (A) 5%, (B) 10%, (C) 15% and (D) 20% in PLLA.

Nanomaterials can improve material properties but overall nanotoxicity has not yet been evaluated; however, the matrix polymer is biocompatible, while barium sulphate and hydroxyapatite have been used extensively in the body.

Conclusions

With improved mechanical strength and radiopacity, BRS fabricated from the experimented material can have decreased strut thickness, and therefore cause fewer issues with blood flow turbulence.

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Acknowledgments

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Biodegradable Polymer for Positive Tissue Irritation in Bone Growth Stimulation

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Introduction

The induced membrane technique is a two-stage procedure in widespread clinical use for treating critical-sized segmental bone defects.[1] In the first operation, the entire bone segment is removed and replaced with a poly(methyl methacrylate) (PMMA) spacer. A membrane which secretes cytokines and growth factors supporting bone regeneration then encapsulates the spacer. In a second operation, the spacer is removed and the void inside the membrane is filled with a bone graft supporting subsequent bone healing. We aim at regenerating segmental bone defects with porous bioactive glass scaffolds in a one-stage procedure. Our hypothesis is that a quickly degrading polymeric coating on the scaffold would support the formation of an induced membrane richer in cytokines and growth factors, leading to improved bone formation and defect healing. Here we report how a low-molecular weight poly(lactide-co-glycolide) (PLGA) coating affects properties of the induced membrane, including the RNA expression of cytokines and growth factors.

Experimental Methods

Bioactive glass S53P4 granules were sintered into porous cylindrical rods, which were either left uncoated or one end was dip-coated in dichloro-methane with 20 wt.% PLGA (Purasorb PDLG 5002A, Corbion, the Netherlands). A 6mm wide horizontal defect was drilled in the distal metaphysis of the femur in skeletally mature NZW rabbits. The defect was filled with either PMMA, uncoated S53P4 or PLGA-coated S53P4 or left empty for control. Follow-up was 2, 4 or 8 weeks with 3 parallels for each implant type and time point. The induced membrane was recovered and RNA expression of VEGF, TNF-α, BMP-2, BMP-4 and BMP-7 was analysed with RT-qPCR. Capillary count was determined from histological sections. In statistical analysis, p-values <0.05 were considered significant.

Results and Discussion

RNA expression for PLGA-coated scaffolds increased significantly over time for VEGF and BMP-4, with increasing trends for BMP-2 and BMP-7 (Fig. 1).[2,3] RNA expression for uncoated scaffolds tended to peak at 4 weeks, whereas for PMMA the expression tended to be stable or decreasing over time. Capillary count for PLGA-coated S53P4 was significantly higher than for PMMA at 8 weeks.

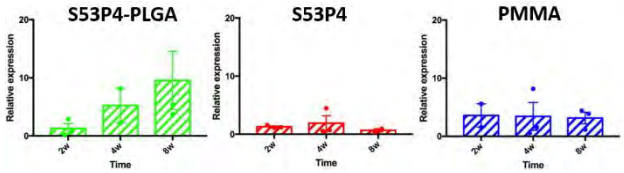

Figure 1. RNA expression of BMP-7 in the induced membrane for PLGA-coated S53P4, uncoated S53P4 and PMMA after 2, 4 and 8 weeks.

The bone regeneration process is longer than the 8 weeks that was tested here. It appears that the PLGA-coated scaffolds did induce membranes which would better support bone formation than those induced by uncoated scaffolds or PMMA.

Conclusions

A quickly degrading polymeric coating which causes irritation to the surrounding tissue may be beneficial for initiating or intensifying biological processes that augment tissue regeneration.

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Acknowledgments

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Hydrolysis of Photo-crosslinked Poly(trimethylene carbonate)-anhydride Networks in Phosphate Buffered Solution

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Introduction

Photo-crosslinked poly(trimethylene carbonate) (PTMC) networks are tough, flexible and degrade via enzymatic surface erosion[1]. The degradation is slow with a 3.7% mass loss in 36 week *in vivo*. Previously, our group worked on poly(ester anhydrides) that degrade hydrolytically in 48-72 hours[2]. We hypothesized that adding a labile anhydride bond to hydroxyl terminated PTMC oligomers and subsequent methacrylate functionalization would result in rapidly degrading photo-crosslinked PTMC-anhydride networks. In this study we report on the preparation of these polymers and the degradation of the networks.

Experimental Methods

Two, three-armed, hydroxyl-terminated PTMC oligomers with a molecular weight of 2000 g/mol were prepared by ring opening polymerization. One of the oligomer was acid-functionalized in a reaction with succinic anhydride. Both oligomers were then methacrylate-functionalized to obtain photo-crosslinkable macromers.

The macromers were mixed with photo-initiator and photo-crosslinked in an in-house constructed crosslink box at 395-405 nm, 1 mW/cm^2 for 30 minutes and subsequently post-cured under visible light for 40 minutes. The gel content was determined in chloroform. Degradation was determined by immersing the samples in phosphate buffered solution (pH 7.4) and recovery at pre-determined intervals. Both gel content and degradation were analyzed by measuring the mass of the samples. All measurements were performed in triplicate. Data is shown as averages ± standard deviation.

Results and Discussion

The gel content of all networks was ≥ 97%. Thus, any obtained mass loss during a degradation experiment can be attributed to degradation and are not the result of dissolution of macromere chains that are not part of the network. From Figure 1 it can be seen that the PTMC-methacrylate networks do not hydrolytically degrade in the time-frame used for these experiments as was expected and is in line with the previously reported PTMC network degradation[1]. The PTMC-anhydride networks however, lose 72% of the mass in 48h as a result of hydrolytic degradation.

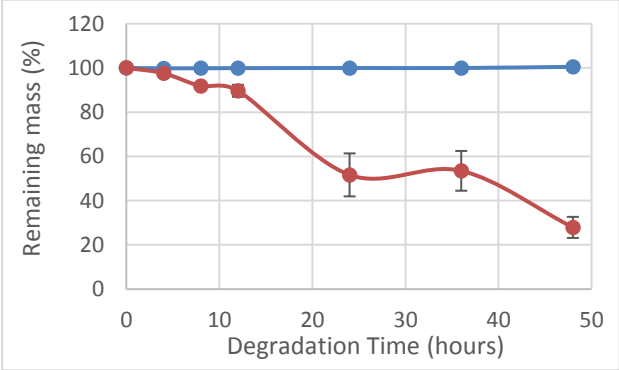

Figure 1. Remaining mass of PTMC networks (blue) and PTMC-anhydride networks (red) in phosphate buffered solution. Values are averages ± standard deviation.

Conclusions

The inclusion of a labile anhydride group in the PTMC network resulted in a hydrolytically degradable photo-crosslinked PTMC-anhydride network. Such networks are interesting for biopolymer applications which require fast degradation.

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Acknowledgments

This work made use of Aalto University Bioeconomy Facilities.

Structure and Dynamics of Thermosensitive DNA Polyplexes Studied by Time-Resolved Fluorescence Spectroscopy

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Introduction

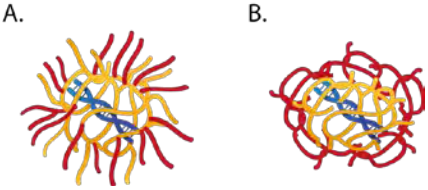
Combining multiple stimuli-responsive functionalities into the polymer design is an attractive approach to improve nucleic acid delivery. However, more in-depth fundamental understanding how the multiple functionalities in the polymer structures are influencing polyplex formation and stability is essential for the development of such delivery systems. In this study, the dynamic nature of thermosensitive polyplexes was investigated by tracking the behavior of labeled pDNA and polymer with time-resolved fluorescence spectroscopy using fluorescence resonance energy transfer (FRET).[1]

Experimental Methods

We developed a synthesis method for controlled polymerization of cationic and thermosensitive linear ABC polymers with PEG as mid block[2] enabling fluorescent labeling of the chain ends. The ABC polymers consisted of thermosensitive *N*-isopropylacrylamide (PNIPAM, N), hydrophilic PEG (P) and cationic 2-(dimethylamino)ethyl methacrylate (PDMAEMA, D) blocks, further referred to as NPD. Polymer block D-chain ends were labelled with Cy3, while pDNA was labeled with FITC. Time-resolved fluorescence was measured with a time-correlated single-photon counting system.

Results and Discussion

The structures of NPD polyplexes showed to be dynamic, meaning that polymer molecules can exchange between core and shell parts resulting in a uniform redistribution of polymer molecules within the polyplex. In contrast to PEI and PLL polyplexes, for which the fluorescence lifetime changes saturate during polyplex shell formation[1], no saturation was observed for NPD polyplexes. The triblock structure of NPD polymers likely resulted in more flexibility compared to polyplexes formed by cationic homopolymers (Scheme 1). The NPD-based polyplexes behaved similarly both at 4 and 37 °C, which is below and above the polymer's cloud point. However, changing the temperature back and forth resulted in irreversible changes and did not yield polyplexes with their initial properties.



Scheme 1. Suggested polyplex structure for studied NPD polymers (A) compared to the structure of the polyplex formed with PEI or PLL (B). Yellow and red indicate core and shell polymers, respectively.

Conclusions

Time-resolved fluorescence spectroscopy combined with fluorescence resonance energy transfer (FRET) is a powerful method for unravelling the structural dynamics of various types of DNA polyplexes. We have revealed the dynamic nature of the structure of thermosensitive polyplexes, which is favorable in the context of DNA release and therefore improved transfection efficiency.

References

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