AFPM

Advanced Functional Polymers for Medicine 2018

May 16th - 18th, 2018
University of Montpellier
France

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Contents

Preface

Dear Delegate,

It is our pleasure to host the Advanced Functional Polymers for Medicine 2018 conference in Montpellier, France.

The purpose of the AFPM conferences, which have been organized since 2010, is to strengthen the interactions within the community of chemists, material engineers, physicists, biologists and clinicians in the development of Advanced Functional Polymers for Medicine.

The 2018 edition of the conference features scientific sessions on the following topics:

- Polymeric biomaterials for advanced therapeutic delivery
- Cells/biomaterials interactions
- Processing of polymeric biomaterials for medicine
- Natural and synthetic functional polymers for medicine
- Advanced Polymers for Medicine: an industrial perspective

The scientific program comprises 29 plenary lectures by experts in their respective fields. In addition, a poster session with 40 presenters provides a comprehensive overview of the latest research on polymeric materials having innovative functionalities for medicine.

Montpellier is an ideal place for our gathering. With approximately 60000 students, Montpellier is a vibrant university city located on the Mediterranean coast in the South of France. Established in 1289, the University of Montpellier is one of the oldest in the world, with the renowned Faculty of Medicine being the world's oldest medical school still in operation. The historical centre Écusson, with its maze of old alleyways and narrow streets, is packed with elegant town houses, trendy stores and plenty of bars and restaurants. Be sure to find a moment after the scientific sessions to ‘get lost’ during your exploration of Montpellier and enjoy a drink on one of the many terraces under the Mediterranean sun!

The AFPM 2018 conference could not have been organized without the generous support of our sponsors: Medincell, PCAS, La Région Occitanie, Montpellier Université d’Excellence, IOP Publishers, Pôle Chimie Balard, Shimadzu, Apidel and CNRS.

Thank you for coming to the AFPM 2018 conference!

Benjamin Nottelet

Program

Wednesday May 16th

10:30	**Registration**	
12:00	**Get together lunch**	
13:00	**Welcome and introduction to AFPM 2018**	
	Oral session 1: Processing of polymeric biomaterials for medicine	
13:30-14:00	Catherine Picart INP Grenoble, France	Active coatings based on biopolymers for tissue regeneration
14:00-14:30	Luigi Ambrosio IPCB Naples, Italy	Cell instructive nanofibers based platforms for tissue regeneration/repair
14:30-15:00	Niels Larsen Technical University of Denmark	Microengineering polymer properties in full 3D for organ modeling
15:00-15:30	Eugenia Kumacheva University of Toronto, Canada	Nanofibrillar hydrogels with a spectrum of properties
15:30	**Coffee break**	
	Oral session 2: Natural and synthetic functional polymers for medicine	
16:00-16:30	Tina Vermonden University of Utrecht, The Netherlands	Native chemical ligation in flower like and golden thermosensitive micelles
16:30-17:00	Christine Jérôme University of Liège, Belgium	Reversible reactions to the service of reprocessable shape memory polymers
17:00-17:30	Anna Finne-Wistrand KTH Stockholm, Sweden	Degradable polymers with time-dependent mechanical characteristics or influenced by deformation strain contribute to improved tissue regeneration
17:30-18:00	Robert Luxenhofer University of Würzburg, Germany	Poly(2-oxazoline)s amphiphiles: From ultra-high loaded micelles to 3D-bioprinting
18:00-20:00	**Poster session & drinks**	
20:00	**Dinner at the Trinque Fougasse O'Sud wine bar**	

Thursday May 17th

	Oral session 3: Advanced Polymers for Medicine: an industrial perspective	
09:00-09:30	Grégoire Schwach Roche, Switzerland	Recent advances in the field of ocular long-acting delivery
09:30-10:00	Robert Gurny Apidel, France	Improved local delivery using micelles - possibilities and limitations
10:00-10:15	Margaret Donnelly IOP Publishers, Washington, USA	Introduction to IOP Publishing's journals Multifunctional Materials (MFM) and Biomedical Materials (BMM)
10:15	**Coffee break**	
11:00-11:30	Ghislaine Barouti PCAS, France	PLGA copolymers: update on clinical trials and industrial challenges
11:30-12:00	Murielle Oster MedinCell, France	BEPO™: injectable *in-situ* forming depots for the sustained release of active pharmaceutical ingredients
12:00	**Lunch & poster session**	
	Oral session 4: Polymeric biomaterials for advanced therapeutic delivery	
13:30-14:00	Julio San Román ICTP Madrid, Spain	The self-assembling of nanoparticles controlled by polymer microstructure. Application as bioactive nanocarriers in regenerative medicine
14:00-14:30	Sébastien Lecommandoux LCPO Bordeaux, France	Biomimetic and multi-functional polymersomes for (nano)medicine
14:30-15:00	Maria Vicent CIPF Valencia, Spain	Versatile crosslinked star-shaped polypeptide conjugates with controlled self-assembly as therapeutics
15:00	**Coffee break**	
	Oral session 5: Cells/biomaterials interactions	
15:30-16:00	Abhay Pandit National University of Ireland	Redefining identity of disease, tissues and cells - a biomaterials paradigm
16:00-16:30	Elisabeth Engel IBEC Barcelona, Spain	Creating microenvironments for tissue regeneration
16:30-17:00	Julien Gautrot Queen Mary University London, UK	Mechanisms of cell sensing of the nanoscale topography and mechanics of the ECM
17:00-17:30	David Eglin AO Research Institute Davos, Switzerland	Stimuli-responsive polymers for manipulation of the cellular environment
17:30	**Conference photo**	
19:15	Gathering in front of the conference venue for the bus trip to Palavas	
20:00	**Gala dinner at the lighthouse of Palavas (buses leave for Montpellier at 23:00)**	

Friday May 18th

	Oral session 6: Processing of polymeric biomaterials for medicine	
09:00-09:30	Jukka Seppälä Aalto University, Finland	3D-Printed biopolymer scaffolds for tissue regeneration and nerve guiding
09:30-10:00	Philippe Poulin Centre de Recherche Paul Pascal, Bordeaux, France	Shape and temperature memory of polymer nanocomposites
10:00-10:30	Dirk Grijpma University of Twente, The Netherlands	Novel biodegradable thermoplastic polyurethane hydrogels for 3D printing
10:30	**Coffee break**	
	Oral session 7: Polymeric biomaterials for advanced therapeutic delivery	
11:00-11:30	Twan Lammers RWTH Aachen, Germany	Polymeric nano- and micromaterials for drug targeting to tumors
11:30-12:00	Gennara Cavallaro University of Palermo, Italy	Tailor-made functionalized polymers for nanomedicine applications
12:00-12:30	Tomáš Etrych Academy of Sciences Prague, Czech Republic	Polymer-based nanomedicines as a tool for personalized tumor treatment
12:30	**Lunch & poster session**	
	Oral session 8: Natural and synthetic functional polymers for medicine	
14:00-14:30	Andreas Lendlein HZG Teltow, Germany	Reprogrammable soft shape-memory actuators with the option of degradability and self-healing
14:30-15:00	Didier Letourneur University Paris Diderot, France	Polysaccharides for imaging and treatment of cardiovascular diseases
15:00-15:30	Benjamin Nottelet University of Montpellier, France	Functional degradable polymers for advanced applications in clinical imaging
15:30	**Poster award and closing remarks**	
16:00	**Goodbye coffee**	

Practical information

Conference venue
Maison des Étudiants “Aimé Schoenig”
Bât. B – Espace Richter
Rue Vendémiaire – CS 19519
34960 Montpellier

The venue is within walking distance from Montpellier’s historical centre (20 min walk). The closest tram stop is ‘Rives du Lez’ (line 1).

Social events (included in conference fee)
Dinner on Wednesday May 16th (20:00)
Trinque Fougasse O’Sud
148 rue de la Galata
34000 Montpellier

Gala dinner on Thursday May 17th (20:00, buses leave from the conference venue at 19:30)
Restaurant Le Phare
Place de la Méditerranée
34250 Palavas-les-Flots

Lecture abstracts

Bone regeneration via osteoinductive polymeric coatings

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In vivo, cells are surrounding by an extra-cellular matrix (ECM) which provide them with bioactive signals coming for the ECM proteins and growth factors. Presenting active biomolecules to cells in a spatially controlled manner to cells using biomaterials as carriers enable to mimic some aspects of the native ECM and to study cell signaling and tissue formation. Surface coatings made of biopolymers [1, 2] can be engineered to trap the bone morphogenetic proteins and present the BMPs to cells at their ventral side. In vitro, this matrix-bound presentation of growth factor to cells via a biomimetic coating reveals so far hidden phenomena [3] [4] and, in the case of bone morphogenetic proteins (BMPs), induces a potent formation of bone tissue formation [5, 6]. These biomimetic 2D surface coatings deposited on a 3D hollow polymeric tube can successfully repair, in a dose-dependent manner, a critical size bone defect in rat femurs [7].

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Cell instructive nanofibers based platforms for tissue regeneration/repair

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Introduction

A variety of additive technologies (i.e., 3D printing, electrofluidodynamics) are currently *in vitro* investigated to reproduce all functionalities exerted "in vivo" by health or pathological tissues microenvironment [1]. In this context, electrospinning represents a highly flexible and low-cost process able to manipulate biomaterials by utilizing electrostatic forces, to design fibers/particles at micro and/or sub-micrometric size scale for different biomedical applications. By combining synthetic/natural polymers and/or active molecules, variously assembled by tailored experimental setups, it allows generating a plethora of 3D scaffolds with peculiar topological or biochemical signals able to trigger different cell activities [2]. Here, we overview current strategies in the use of electrospinning alone - or in combination with electrospraying (AES) [3] - to develop cell instructive scaffolds for TE.

Experimental Methods

PCL and PCL/Gel fibres were produced by using a commercial equipment (Nanon01, MECC). As For AES, AMX or TCH loaded Chitosan (CS) nanoparticles were optimized. Morphology was investigated by SEM/TEM/AFM microscopy. Drug release was estimated by UV-VIS spectrophotometry. The antibacterial activity was evaluated by disc diffusion, measuring the inhibition zones into the agar plate. Different cell (i.e., hMSC, neurons, cardiomyocytes) response was investigated by different cell assays.

Results and Discussion

We demonstrated that PCL electrospun fibres with peculiar morphological properties may support the regeneration processes in the skeletal system (i.e., bone, nerve, skeletal muscle) [5-7]. The combination of PCL with Gelatin allows better mimicking native ECM microenvironment by imparting peculiar biochemical signals able to improve *in vitro* cell recognition [9], also carrying out bioactive molecules (i.e., drugs) by controlling *in vitro* depletion [10]. Alternatively, the use of electrospinning in combination with electrospraying allows designing more complex platforms able to retain active molecules (i..e, growth factors, antibiotics) much longer than electrospun fibres alone.

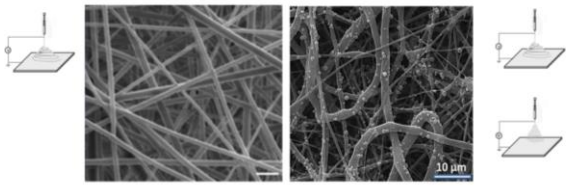

Figure 1: cell instructive scaffolds via electrospinning (left) and AES (right) techniques**;**

we have verified that AES may be optimized to decorate PCL nano fibers with AMX loaded chitosan (CS) nano-reservoirs to release antibacterial drugs in oral cavity [13]. Meanwhile, TCH may be efficiently trapped in CS particles to provide a more prolonged and efficient drug administration in the presence of inflammation of the periodontal pocket [14].

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Acknowledgments

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Microengineering Polymer Properties in Full 3D for Organ Modeling

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Introduction

3D printing of hydrogels is being explored to enable advanced cell culture under tissue-like conditions due to the high oxygen and nutrient permeability of compliant hydrogels.[1] However, fragile hydrogels are not easily interfaced to much stiffer tubing required for microperfusion. Culture system manufacture and integration would benefit from access to materials and processing methods where the mechanical properties can be tuned locally from compliant to stiff during manufacture.

Experimental Methods

Light-guided 3D printing (stereolithography) is promising for high-resolution shaping of both stiff and compliant materials.[1] However, no reported material and method can vary the hardness broadly within a printed object without complex transfers between multiple precursor baths, leading to slow print speeds, complex printing systems, and limited spatial resolution. We have met this challenge by combining spectrally independent radical-initiated photopolymerization of poly(ethylene glycol)-diacrylate monomers (compliant) and cation-initiated polymerization of epoxy monomers (stiff) on a custom-build stereolithographic printer capable of spatially aligned exposure at multiple wavelengths.

Results and Discussion

The local compression moduli of the resulting polymer material can be varied in the range from roughly 200 kPa to 20 MPa by illuminating at two distinct wavelengths (365 nm and 450 nm) for defined times (Figure 1).[2] We have further demonstrated 3D printing of fully connected acrylate- and epoxy-based materials to form mechanically robust microperfusion systems.

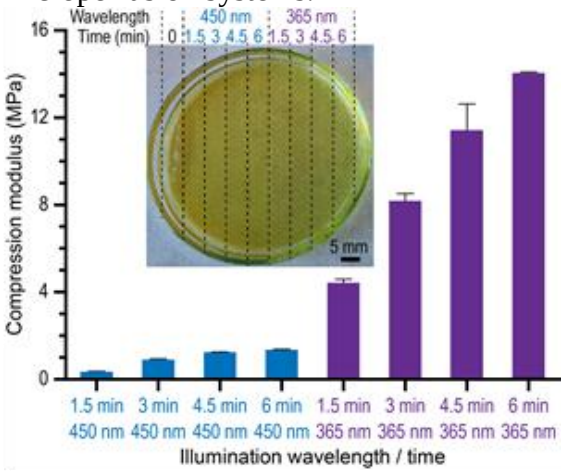

Figure 1. Compression modulus across a Ø60 mm hydrogel disk (insert) after exposure of an acrylate / epoxy monomer mixture solution to 365 nm or 450 nm light for different times.

Conclusions

The materials and 3D printing platform provides useful mechanical properties and spatial detail for microengineering the cell environment and for direct fluid interfacing to external perfusion and analysis systems.

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Acknowledgments

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Nanofibrillar Hydrogels for Cancer Spheroid Growth

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Synthesis and fabrication of man-made hydrogels mimicking natural extracellular matrix is an important and challenging task. We developed cellulose nanocrystal (CNC)-derived nanofibrillar hydrogels and used them as scaffolds for cell culture. One example of such scaffold is a supramolecular temperature-responsive hydrogel formed by CNCs tethered with polymer molecules. A filamentous hydrogel was formed at 37◦C from an aqueous CNC suspension and used for cell encapsulation and growth. On demand, the cells were released by lowering the temperature and subsequently transferred to another scaffold. The second hydrogel was formed by covalent crosslinking of gelatin and CNCs carrying surface aldehyde groups. By using 3D printing, we generated structurally anisotropic hydrogel with gradients in composition, structure and properties.

Native Chemical Ligation in Flower Like and Golden Thermosensitive Micelles.

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Introduction

Thermosensitive polymeric micelles are attractive as drug delivery vehicles because of their reversible self-assembling nature as a function of temperature. However, to prevent rapid micelle dissociation in vivo, chemical crosslinking techniques are often used to enhance stability and obtain long circulating nanoparticles. Native chemical ligation (NCL) is an appealing method to covalently crosslink polymers, because of its ability to react under physiological conditions avoiding the use of toxic reagents and catalysts, making this method very biofriendly.[1] Since NCL is a very specific ligation between *N*-terminal cysteines and thioesters, side reactions with biomolecules can be ruled out and, therefore, NCL is expected to be highly compatible with encapsulated drugs. In this study, NCL core-crosslinked, thermosensitive, flower like micelles and starlike, gold nanocluster containing micelles were investigated for drug delivery applications.

Experimental Methods

ABA triblock and AB diblock copolymers consisting of polyethylene glycol (PEG) as B-block and thermosensitive poly isopropylacrylamide (PNIPAM) A-blocks decorated with either cysteine P(NIPAM-co-HPMA-Cys) (PNC) or thioester P(NIPAM-co-HPMA-ETSA) (PNE) functionalities were synthesized by atom transfer radical polymerization (ATRP) or reversible addition-fragmentation chain transfer (RAFT) polymerization. Aqueous solutions of these complementary polymers were mixed at 4 ºC and rapidly heated to form either flower-like (ABA) or star-like (AB) micelles. Thiolated doxorubicin (Dox-SH) was prepared according to a literature procedure.[2] $HAuCl_4$ was reduced in the presence of micelles and Dox-SH to obtain gold nanocluster decorated micelles

Results and Discussion

Mixing of the two complementary ABA polymers PNE and PNC in aqueous solution followed by heating to 50 ºC resulted in the formation of thermosensitive, flower like micelles. Subsequently, native chemical ligation in the core of micelles resulted in stabilization of the micelles with an average diameter of 65 nm at 37 °C. Decreasing the temperature to 10 °C only affected the size of the micelles (increased to 90 nm) but hardly affected the polydispersity index (PDI) and aggregation number (N_{agg}) confirming covalent stabilization of the micelles by NCL. Notably, by simply adjusting the molar ratio between the polymers, the extra cysteine or thioester moieties could be used for conjugation of functional molecules. Furthermore, in vitro cell experiments demonstrated that fluorescently labeled micelles were successfully taken up by HeLa cells, while cell viability remained high even at high micelle concentrations.[3]

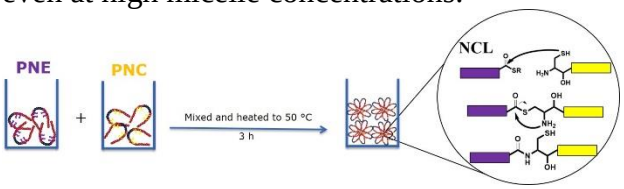

Figure 1. PNC and PNE were dissolved separately in buffer at 4 °C and subsequently mixed in a 1:1 ratio and immediately heated up to 50 °C. The micellar solution was left at 50 °C for 3 h to let native chemical ligation proceed in the micellar core.

Analogous star-like micelles were prepared from AB block copolymers having an excess of thiol functionalities, which were used to covalently load gold nanoclusters associated with thiolated doxorubicin. This formulation showed a highly localized killing capacity when irradiated with a 532 nm laser in vitro using MDA-MB-231 breast cancer cells.

Figure 2. Complementary AB polymers containing excess thiol groups form micelles by heating and NCL. Subsequently, gold nanoclusters (yellow dots) with thiolated doxorubicin (red dots) were entrapped.

Conclusions

The use of native chemical ligation as a biofriendly, crosslinking method has shown to be very versatile for not only stabilizing micelles but also efficiently linking fluorescent labels, gold nanoclusters and drugs.

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Acknowledgments

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Reversible reactions to the service of reprocessable shape memory polymers

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Introduction

Shape-memory polymers (SMPs) are remarkable materials able to switch from a temporary shape to their initial permanent shape by crossing a thermal transition, e.g. glass or melting transition. Efficient shape-memory effect is notably observed for chemically cross-linked semi-crystalline polymers. Chemical networks of semi-crystalline poly(ε-caprolactone) (PCL) are widely studied for the development of SMPs especially when biomedical applications are foreseen. As these SMPs are irreversibly cross-linked materials, their (re)processing is quite limited since they cannot be molten or solubilized after cross-linking. This prevents any recycling. Thereby, by using reversible cross-linking reactions to allow the formation or cleavage of the network upon a selected stimulus (see scheme), raise tremendous interest for the development of smart SMPs.

Results and discussion

Chemical but remandable networks of PCL were designed by coupling PCL stars via reversible cycloaddition reactions. The thermo-reversible Diels-Alder reaction between furan and maleimide [1-2] was investigated to crosslink the PCL matrix, and compared to the photo-induced (2+2) cycloaddition of coumarins and to the Alder-ene reaction of 1,2,4-triazoline-3,5-dione with indole compounds [3]. With these cross-links, the typical shape memory properties of PCL networks (high fixity and recovery) were preserved while upon an external (light or stress) stimulus, the PCL network can be (re)processed efficiently.

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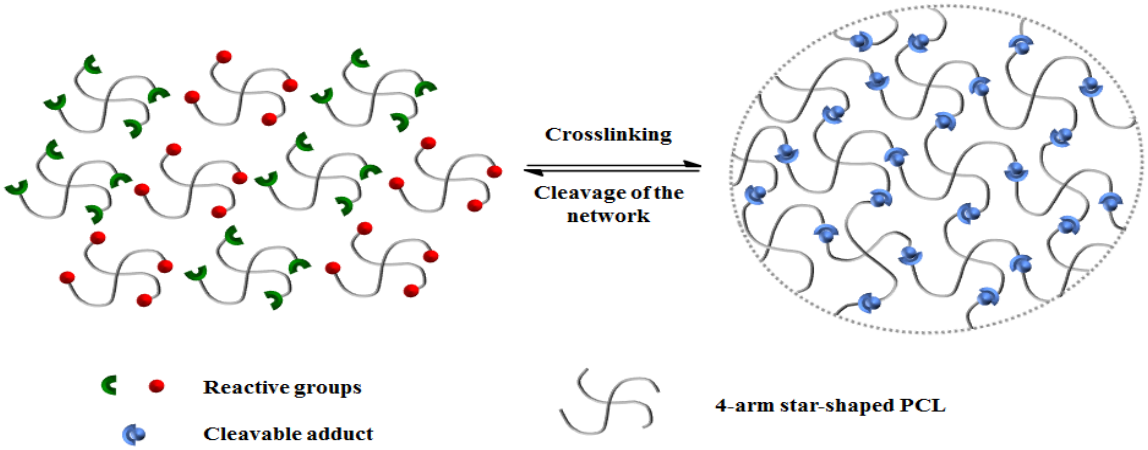

Degradable polymers with time-dependent mechanical characteristics or influenced by deformation strain contribute to improved tissue regeneration

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Introduction

Cells are continuously influenced by a range of mechanical strains, for example fluid-flow-induced shear stress, cyclic stretching and compression. Recognizing such influences of mechanical stimuli on cell phenotype regulation has allowed important advances in tissue engineering. We have evaluated mechanical strain stimulation from two different perspectives 1) preclinical evaluation using material with time-dependent mechanical characteristics[1] 2) in vitro evaluation of degradable polymers influenced by deformation strain.[2,3]

Experimental Methods

1) TIGR® Matrix Surgical Mesh is a macroporous multifilament surgical mesh, knitted from two different synthetic resorbable fibers, possessing different degradation characteristics. In 14 female sheep, a medial incision of the abdominal skin was performed.[1]

2) 3D poly(L-lactide-co-ε-caprolactone) porous scaffolds of 10.5 mm in diameter and 12 mm in length were exposed, in a bioreactor, to uniaxial compression and unloading.[2]

Results and Discussion

1) The mesh gradually degraded and the formed granulation tissue was at the beginning rich in collagen type III, during the wound healing process it was replaced by collagen type I. When compared to a synthetic non-degradable mesh, the ratio collagen type I:collagen type III was higher in the mesh with time dependent characteristics. The degradable mesh was well integrated into fibrous connective tissue and early neovascularization was observed.

2) Compared with a constant flow control conditions, the level of calcification increased significantly when the scaffold was deformed at 1 Hz. The results also revealed that deformation strain of degradable 3-dimensional scaffolds was the predominant stimulus for skeletal precursors to undergo osteogenesis in earlier stages of osteogenic cell maturation.

Conclusions

The results demonstrate the importance of mechanical stimulation in tissue engineering. Mechanical strain using time dependent mechanical characteristics stimulate remodeling of collagen towards a strong connective tissue and deformation strain stimulate early osteogenic cell maturation.

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Acknowledgments

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Poly(2-oxazoline)s amphiphiles: From ultra-high loaded micelles to 3D-bioprinting

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Poly(2-oxazoline)s have been investigated for some time as biomaterials, but remain to have a very large untapped potential.

We have previously reported on a novel polymer platform, which is allows to formulate unparalleled amounts of the extremely water insoluble paclitaxel [1,2,3] and other APIs and API combinations [4]. More recently, we have found very pronounced effects of very small structural variations between poly(2-oxazoline)s and poly(2-oxazine)s on the formulation capacity for curcumin and paclitaxel, another highly insoluble natural compound [5]. Similarly, small structural changes significantly affects the polymer properties and their potential applications. For example, a diblock copolymer of poly(2-methyl-2-oxazoline)s and poly(2-n-propyl-2-oxazine)s shows very interesting thermogelation and shear-thinning behavior [6], while the triblock copolymer is an excellent drug solubilizer. Due to the excellent cytocompatibility, this novel hydrogel should be excellently suited as a bioink, as demonstrated in preliminary work using NIH 3T3 cells.

Conclusions

Poly(2-oxazoline) and poly(2-oxazine) based amphiphiles are very divers families of polymers, which are readily combined to provided even larger versatility. Most interestingly, by smallest changes in the polymer structure and architecture, significant changes in the performance and properties can be affected. To date, only a minute fraction of possible polymers in this large cosmos have been explored, which leaves a multitude of opportunities to investigate new avenues.

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Recent Advances in the Field of Ocular Long-Acting Delivery

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Introduction

The eye is a complex organ to target, and there is a large unmet need for longer acting therapies, especially for biologic therapeutics. A drug delivery approach (e.g. long acting, sustained release) has however impact on major product development areas, and difficulties are numerous to overcome.

Experimental Methods

To de-risk extra development effort, experimental predictive ocular models have shown to be useful (1). Examples of long acting technologies currently under investigation at Roche will be discussed as well (PLGA rod implants).

Results and Discussion

Artificial vitreous humor (aVH) was successfully developed (table 1), and was used to assess therapeutic proteins stability in the eye. Data of model mAb showed good comparability between porcine-derived and aVH models, in various experimental configurations. These promising data need confirmation with other molecules and longer durability.

Artificial Vitreous Humor (aVH)							
		Initial	Week 1	Week 2	Week 5	Week 8	Week 12
1	Density	1.0073			1.0073	1.0074	1.0074
2	Osmolality (Osmol/kg)	0.307	0.303	0.300	0.307	0.306	0.308
3	pH	7.15	7.19	7.18	7.19	7.19	7.19
4	Viscosity (mPa.s)	71.5	72.7	74.9	73.4	72.6	63-66

Table 1. Stability of key physico-chemical parameters of aVH.

PLGA rod implants are an attractive drug delivery option for sustained delivery of small molecules to the eye. API investigated was very stable under extrusion, sterilization and in vitro release. The system exhibited relatively constant release rates over up to 5-6 months (Fig 1) without burst release. These data were confirmed in a pilot PK study in rabbits.

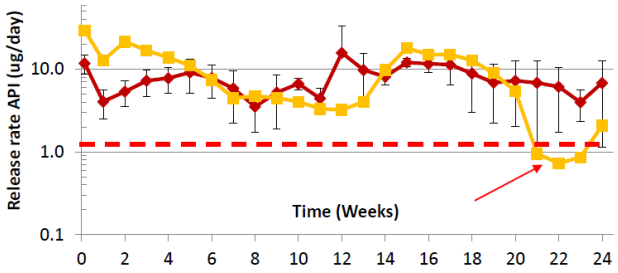

Figure 1. In vitro release of API from PLGA rods made of two formulations (PBS, pH 7.4, 37°C, n=3, sterile rods).

Conclusions

For the successful development of longer acting drugs in ophthalmology, it is key to understand the biology, the barriers and the effects of disease state. Ocular models are valuable tools in product development to assess stability and drug release aspects. PLGA rods might be a suitable system for small molecules. For biologics, a drug-device combination might be preferred.

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Improved local delivery using micelles: Possibilities and limitations

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The concept of the recent development of patient-friendly and efficient treatment of pathologies that need local delivery of therapeutic agents is based on micellar drug delivery systems. The risk of systemic side effects is greatly reduced when using local administration. However, the physico-chemical properties of numerous commonly used drugs such as antifungals, cytostatics or immune modulators make their formulation for local delivery a considerable challenge due to their poor solubility. The objective of the present critical overview is to present the concept and limitations of novel nanostructures (micellar solutions) dispersed on a nanoscale in a hydrogel and loaded with cyclosporine (CsA), clotrimazole (CLZ), econazole nitrate (ECZ), tacrolimus (TAC) and fluconazole (FLZ). These formulations use novel optimized amphiphilic block copolymers based on caprylic acid (GRAS, E570) and PEG (MW 2500). The CsA, CLZ, ECZ, TAC and FLZ formulations were prepared and characterized with respect to stability, drug loading and micelle size. The optimal drug formulations were selected for transport- and pk-studies that were performed on human or animal tissues and animal models. Penetration pathways and micellar distribution were visualized using fluorescent-labeled micelles, confocal laser scanning microscopy or soft X-ray imaging. The hydrodynamic diameters of the loaded micelles were between 15 and 50 nm. Drug incorporation rate is typically over 98.5% in the micelles. As examples, the performance of the delivery from a formulation Pevaryl® cream (1% w/w ECZ), a marketed liposomal formulation for topical application, will be presented compared to the novel micellar formulation. Protopic® ointment for dermatitis (0.1% TAC) will also be challenged by novel nanodispersed formulations. Futhermore ophthalmic formulations (CsA 0.01%) have been prepared and will be compared to Restasis®.

In conclusion, the significant increase in tissue deposition achieved using the novel strategy is clearly demonstrated and the novel concept translates into improved clinical efficacy *in vivo*.

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Introduction to IOP Publishing's journals Multifunctional Materials (MFM) and Biomedical Materials (BMM)

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Introduction

Margaret Donnelly, a Publisher from IOP Publishing, will introduce a newly launched journal, Multifunctional Materials (MFM), and share information on an established journal, Biomedical Materials (BMM; 2016 IF 2.469). Invited speakers and poster presenters at the Advanced Functional Polymers for Medicine will be invited to submit their work to these journals following the conference.

PLGA copolymers: Update on clinical trials and industrial challenges

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The development of biodegradable and biocompatible polymers allowed the conception of innovative technologies in the health area during the last few decades.[1,2] It represented a real revolution for improving drug bioavailability and reducing severe side effects during the treatment of several diseases.

PLA (Poly(lactic acid)) and PLGA (Poly(lactic-co-glycolic acid)) derivatives were rapidly used for the design of drug delivery systems due to their interesting properties such as biocompatibility, biodegradability and drug release capabilities.[3] However, despite the huge interest and potentials of these polymers, the bench-to-market translation remains challenging particularly from manufacturing and regulatory perspectives. This translation requires the production of large scale batches of polymers under Good Manufacturing Practice (GMP) conditions with a very good batch to batch consistency. There are currently less than 20 FDA approved PLA/PLGA based products available on the market. Moreover the development of generic underlines the importance of robust manufacturing process to ensure the production of high quality, safe and efficient product.

PCAS designs and develops a wide range of PLA and PLGA (Expansorb®) based copolymers in order to respond to the pharmaceuticals companies needs.[5] Industrial challenges corresponding of engineering such polymers will be discussed and an update on current clinical trials involving PLA/PLGA based product will be presented to define future perspectives.

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Merck is acknowledged for scientific data concerning formulation with Expansorb® polymers and applications case studies.

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BEPO™: Injectable *in-situ* forming depots for the sustained release of active pharmaceutical ingredients

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Introduction

MedinCell is a French company that develops technologies for the sustained release of active pharmaceutical ingredients (API). The company mission to make medicine affordable to all is at the center of all research and development projects. Its core technology,[1] BEPO™, is based on bioresorbable amphiphilic block copolymers that once mixed with the API and a biocompatible solvent will yield injectable solutions or suspensions, depending on the solubility of the API. Upon injection into an aqueous environment, such as the subcutaneous (SC) tissue, the copolymers will precipitate forming a depot which physically entraps the API. The subsequent release of therapeutic active molecules will be driven by their diffusion and copolymer degradation. Several formulation variables allow to obtain release durations from some days up to a year. BEPO™ is currently being used for producing best-in-class products for a wide range of drugs for the treatment of schizophrenia, depression, pain or contraception and anesthesia; two of these products are already undergoing clinical trials.

Results and Discussion

BEPO™ formulations utilize combinations of (m)PEG-*b*-PLA based di- and triblock copolymers for achieving the sustained release of API. The ratio between the hydrophobic and hydrophilic blocks, the length of each block, the triblock/diblock ratio, the copolymer content or the loading of API can be tuned to ensure plasmatic concentrations within the therapeutic window for a given duration, as depicted in Figure 1. The viscosity and the injectability of the formulations as well as the degradation kinetics of the depots are considered while formulating drug products and can be tailored depending on the foreseen application.

When administered SC, the depot can be located and monitored over time by ultrasounds echography, which enables its exeresis in case of emergency. MedinCell is also developing tools which mimic *in-vivo* SC conditions in order to enhance the predictability of *in-vitro* results during the formulation process.

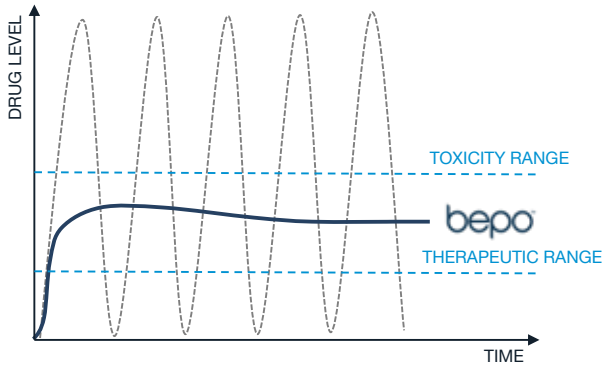

Figure 1. Typical API plasmatic concentrations obtained after a single BEPO™ SC injection vs after regular oral administration.

Conclusions

MedinCell has developed BEPO™, a technology that enables the sustained release of APIs from several days up to a year using (m)PEG-*b*-PLA block copolymers based injectable formulations that form *in-situ* depots upon administration.

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THE SELF-ASSEMBLING OF NANOPARTICLES CONTROLLED BY POLYMER MICROSTRUCTURE. APPLICATION AS BIOACTIVE NANOCARRIERS IN REGENERATIVE MEDICINE

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Relevant advances in the new concept of "Nanomedicine" are based on the design and preparation of polymeric chains with specific hydrophobic or hydrophilic character by the reversible linking of bioactive compounds to macromolecular systems. This is possible by means of the reaction of functional groups present in the macromolecule, or by copolymerization of functionalized bioactive compounds or drugs, with specific polymerizable functions. This biomimetic approach offers interesting designs by selecting polymerization mechanisms and composition of the active monomers, to give high molecular weight polymers with controlled microstructure, composition and morphology. The control of the composition and microstructure of copolymer chains give rise to the formation of self-assembled bioactive nanoparticles that can be applied as nanocarriers of specific drugs, and in this sense the results obtained with the nanocarriers of the acrylic derivative of vitamin E and N-vinyl pyrrolidone loaded with a small amount of dexamethasone or methyl prednisolone, gives very good antioxidant properties, and even more we have found an interesting effect of inhibition of the toxic effect of hearing loss associated to the chemotherapeutic application of cis-platin. The formation of core-shell nanoparticles is easily produced if the corresponding monomers are selected considering that one of them has to present a much higher reactivity than the second. In this way, the average composition of the polymer chains changes with the conversion and at the end results in copolymers with long sequences of the corresponding monomers in a gradient microstructure. If one of them is a functionalized bioactive based monomer, we will have the possibility of the preparation of polymers with specific sequences, structures and morphologies which give rise to self-assembling structures and morphologies in physiological conditions. The self-assembled systems can be applied as low toxicity and high activity "Polymer Drugs" as well as nanocarriers of loaded traditional drugs with limited solubility in physiological conditions.

Results obtained by the application of the loaded nanoparticles in an animal with cisplatin-induced ototoxicity, (which affects to 100% of the clinical patients) will be presented.

Acknowledgments

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Biomimetic and multi-functional polymersomes for (nano)medicine

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Introduction

Polymers represent an important class of organic compounds that are used for many years in the development of biomaterials. Among them, amphiphilic block copolymers are among the most attractive systems for drug delivery applications. We report here an overview on the self-assembly in water of amphiphilic block copolymers into different nanomedicines, mainly focusing on polymer vesicles, also referred as polymersomes, and their applications in loading and controlled release of both hydrophilic and hydrophobic molecules and biomolecules.

Results and Discussion

We pay special attention to polysaccharide and polypeptide-based block copolymer vesicles and their development in nanomedicine. Indeed, the field of synthetic polypeptides has seen many significant advances in recent years, including studies on block and hybrid copolypeptides that form vesicles, fibrils, and other structures with potential applications in medicine and materials chemistry. However, the development of glycosylated polypeptides has not kept pace, primarily due to the inability to readily synthesize glycopolypeptides in a controlled manner. Glycosylation of natural proteins provides diverse functionality such as mediation of recognition events, modification of protein conformations, ect, that may find interest and application in biomedical field. In this context, we developed over the last years synthetic strategies for the design of glycosylated polypeptides and polysaccharide-polypeptide biohybrids with controlled placement of sugar functionality. We were especially interested in designing amphiphilic copolymers able to self-assemble into well-defined micelles and vesicles that can advantageously be loaded with drugs and present a surface with multivalent presentation of bioactive saccharides or oligosaccharides. The ability of these nanoparticles for different biomedical applications, from drug-delivery to inhibitor, will be presented. We especially evidenced the particular benefit of nanoparticles and their multivalency toward the interaction with biological receptors.

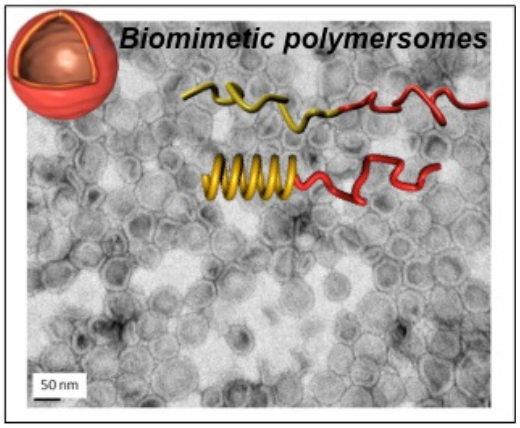

Figure 1. schematic representation and TEM image of polysaccharide-*b*-polypeptide based block copolymer vesicles.

Finally, our recent advances in using "biomimicry approaches" to design complex, compartmentalized and functional protocells will be proposed. Such a system constitutes a first step towards the challenge of structural cell mimicry and functionality, and may act in the future as an autonomous artificial cell that can sense and cure *in situ* any biological deregulation

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Versatile Crosslinked Star-shaped Polypeptide Conjugates with Controlled Self-assembly as Therapeutics

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Introduction

Polypeptides are already playing a major role on a number of different relevant areas such as nanomedicine [1]. The physico-chemical parameters of a polypeptide-conjugate, and hence its biological performance, are defined by an intricate interplay of multiple structural factors. This highlights the need for detailed structure-activity relationship studies to develop the hierarchical strategies of polypeptide conjugate design. However, structural complexity also represents a unique opportunity, since small changes at the structural level might endow nanomedicines with outstanding and unexpected biological performance [2].

Results and Discussion

In our group, we have overcome the main classical limitations for the synthesis of defined polypeptides using precise controlled reactions followed by an adequate characterization yielding to well-defined polypeptidic architectures (including stars, graft and block-copolymers) by NCA polymerization techniques [3]. In addition, post-polymerization techniques allow us the introduction of a variety of functionalities yielding a set of orthogonal reactive attachment sides [4]. Using these techniques and following a bottom-up strategy we have been able to obtain star-based polypeptide architectures with the capacity to self-assemble yielding supramolecular nanostructures with interesting properties [5].

Star-shaped polypeptides with different cores and varied length of arms have been studied. We observed two different mechanism that control the self-assembling behaviour of these polymers. For compounds with short arms we observed formation of supramolecular polymers driven by hydrophobic interactions and hydrogen bonding. For bigger polymers we observed core-independent self-assembly. Supramolecular polymers and polyions with transition state formed distinct morphological structures - fibrillar and spherical, respectively. Interestingly enough, for some molecules we also observed an intermediate mechanism. Many compounds were found to be ionic strength and temperature dependent that directly correlated with the reported mechanism of self-assembly.

This strategy enabled *in vitro* and *in vivo* evaluation, revealing a lack of toxicity, an enhanced *in vitro* cell internalization rate and significantly greater terminal and accumulation half-life *in vivo* together with a significant lymph node accumulation [5].

Conclusions

These results allow us to envisage these systems as promising nanocarriers for therapeutic or diagnostic applications, especially in anti-cancer treatments. Additionally, further studies to identify the mechanism for the significant accumulation found in the lymph nodes will open up a wide range of opportunities for the currently unsuccessful clinical approaches to target lymph node metastasis, imaging of sentinel lymph node and cancer immunotherapy.

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Redefining Identity of Disease, Tissues and Cells – a *Biomaterials* Paradigm

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Biomaterials are no longer considered innate structures and using functionalisation and biofabrication strategies to modulate a desired response whether it is a host or implant is currently an important focus in current research paradigms. Fundamentally, a thorough understanding the host response will enable us to design proper functionalisation and biofabrication strategies. The input from the host response needs to be weighed in depending on the host disease condition. In addition, biomaterials themselves provide immense therapeutic benefits which needs to be accounted in the design paradigm. Using functionalisation strategies such as enzymatic and hyperbranched linking systems, we have been able to link biomolecules to different structural moieties. The programmed assembly of biomolecules into higher-order self-organized systems is central to innumerable biological processes and development of the next generation of biofabricated scaffolds. Recent design efforts have utilized a developmental biology approach toward both understanding and engineering supramolecular protein assemblies. Structural moieties have taken a variety of different forms such as nanofibers and nanoparticulate. This approach has resulted in functionalisation of micro and nanoparticles with biomolecules that include designed peptide motifs, growth factors and a multitude of gene vector systems. In addition, nature itself has abundant structural complexity that can be biofabricated for harnessing in key targeted clinical applications.

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Creating microenvironments for tissue regeneration

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Introduction

Polymers, either natural or synthetic, have been used for regeneration for a long time. Similar polymers as polyesters have been used for different applications with dissimilar results. It depends not only in the selection of the raw material but also in the processing method. Understanding how cells interact with the polymer and how the degradation products can activate metabolically the cells allows the creation of microenvironments that can induce cell reprograming and activation of stem cells. In this talk, I would like to show how different cells are susceptible to change their phenotype and function in response to the designed scaffolds. The selected material, topography, scaffold structure and degradation products have to be optimized to fulfil the requirements for each tissue (1,2). We have demonstrated that biomaterials themselves can activate stem cells at their niche and can either differentiate them or dedifferentiate adult cells towards a more precursor phenotype. This is perfect for in situ tissue engineering applications.

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Mechanisms of cell sensing of the nanoscale topography and mechanics of the ECM

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The mechanical properties and nanotopography of the extracellular matrix have an important impact on cell phenotype. Such physical cues been shown to regulate cell adhesion and spreading, cell motility, proliferation and differentiation in a wide range of cells, stem cells as well as cancer cells. However, mechanisms underlying mechanical and nanotopography sensing remain unclear. We are interested in developing nanoscale engineered extra-cellular matrices to study such mechanisms.

In particular, we show that focal adhesions, typically regarded as essential mechanosensors, are not primary sensors of the nanotopography and that the dynamics of the microscale acto-myosin network acts instead as a global sensor of nanoscale topography and geometry.

In contrast, our results demonstrate that mechanosensing occurs primarily at the nanoscale: we show that cells can directly sense the nanoscale mechanics of their environment. Indeed, we made the surprising observation that adherent cells can spread and proliferate at the surface of low viscosity liquids. Although this does not seem to agree at first glance with the general vue that bulk mechanical properties of materials are essential to sustain focal adhesion maturation, cytoskeletal assembly and cell spreading, we discovered that cell adhesion to liquid substrates is mediated by the assembly of a protein nanosheet, at the interface between the two liquids (an oil and the tissue culture medium). The strength of these nanosheets, depending on parameters regulating its assembly, can sustain shear forces generated by cells during their spreading.

We show that cell spreading at such liquid interfaces is mediated by integrin ligation, focal adhesion formation and acto-myosin contractility. In addition, we show that this behaviour depends on the interfacial mechanical properties of the protein layer assembled. Finally, we show that keratinocyte differentiation is not initiated by spreading at the surface of liquids, despite the absence of bulk mechanical properties. 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.

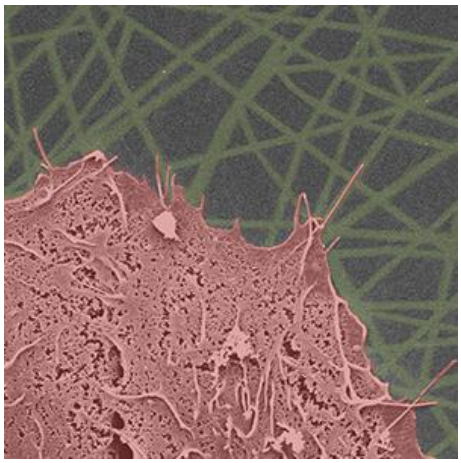

Figure 1. Cell interacting with a network of nanofibers.

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Acknowledgments

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Stimuli Responsive Hydrogels for Cellular Microenvironment Engineering

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Introduction

Stimuli responsive hydrogels are being developed for use as two and three-dimensional extracellular microenvironments to mimic the features of the extracellular matrices (ECM), for basic and translational studies. The polymers developed for such application can be biopolymer-derivatives showing responsivity to environmental factors such as temperature, enzyme and light. These external stimuli control the polymeric network formation *and in fine* the ECM-like environment.
However, there is often a lack of understanding on how the external stimuli and crosslinking mechanism of these derivatives can influence the cells behavior. In addition, anisotropy in ECM mimic matrices are seldom achieved in controlled manner and in a cell compatible way. Enhance, we investigated the role of substrate crosslinking mechanisms, enzymatic and light, on the spreading of mesenchymal stem cells (MSC) on the surface of a tyramine derivative of hyaluronan. We also report a method to produce anisotropic stimuli responsive composite hydrogels based on magnetically responsive poly(ethylene glycol) capped iron oxide nanoparticles / gelatin methacrylate hydrogel.

Results

At a constant Young`s modulus of 7 kPa, MSC spreading area was increased on enzymatically (HRP) crosslinked substrates relative to light crosslinked (EO) matrices (Fig 1). MSCs seeded on anisotropic composite hydrogels aligned parallel to the anisotropy (Fig 2).

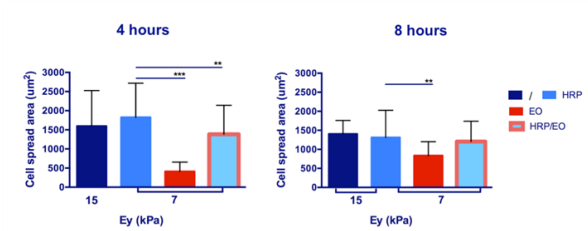

Figure 1. Quantification of MSCs spread area after 4 and 8 hours (mean +/- SD,**p<0.01, ***p<0.001 ANOVA).

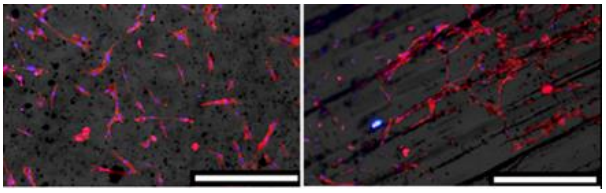

Figure 2. Fluorescent image of MSCs seeded on top of isotropic (left) and anisotropic (right) stimuli responsive composite hydrogels at 24 hours.

Conclusion

These findings highlight the importance of considering the crosslinking chemistry in hydrogel and the ability to use stimuli responsive hydrogels for engineering of cellular microenvironments.

Acknowledgments

M. D'Este, T. Serra, R. Tognato and M. Alini from the AO Research Institute Davos; C. Loebel, R. Mauck from UPenn, US; M. Zenobi-Wong from the ETH-Zurich, CH; R. Levato from Utrecht University, NL; G. Giancane, from Università del Salento, It.

3D-Printed Bio-Polymeric Scaffolds for Tissue Regeneration and Nerve Guiding

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Additive manufacturing of polymers and bioactive composites opens up new avenues for precisely designed and customized biomaterials. Stereolithography is based on the principle of photocuring pre-polymers in pre-designed voxel-areas. Stereolithography was applied in two cases to produce scaffolds for tissue regeneration.

In Case 1 designed tricalcium phosphate (TCP) filled scaffolds were fabricated using stereolithography by photocuring poly(trimethylene carbonate) (PTMC). Filler contents up to 40% particle form TCP could be successfully used in stereolithography. Properties of thus created biomaterials will be presented.

In Case 2 photocuring polycaprolactone scaffolds were designed and 3D-manufactured for nerve guiding application. The printed conduits were filled with hydrogel and then used in cell cultivations and animal studies with encouraging indications of nerve regeneration.

Strong and smart shape memory nanocomposite polymer fibers

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Shape memory polymers are promising materials for various medical applications, including stent, drug delivery systems, and wound healing. They can be easily processed and display large deformations in response to temperature stimuli. We discuss how the memory properties of shape polymer fibers can be improved and enriched by the addition of carbon nanoparticles, including carbon nanotubes and graphene platelets, embedded in the microfibers. We show in particular that these nanocomposite materials exhibit significantly improved energy density of actuation. This improvement is due to the reinforcement of the fibers by the stiff carbon nanoparticles. In addition to be stronger, nanocomposite fibers can also be made smarter. Indeed, shape memory polymer nanocomposite fibers are electrically conductive and display a conductivity memory linked to the temperature of their processing. Lastly we will show recent progresses related to the development of new shape memory torsional actuators. Graphene reinforced polymer fibers exhibit significant improvements of their shear modulus. As a result, such fibers can absorb a high amount mechanical energy when twisted at high temperature. This energy can be stored by quenching the materials to low temperature. It can be further restored by heating the material above its glass transition temperature. The fiber untwists and acts as a torsional actuator capable of generating an exceptionally high torque associated to a giant energy density. The temperature at which the maximum of energy is released can be tuned by changing the programming conditions. We hope that the present results can motivate further research towards the development of actual devices potentially useful in medical applications.

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Novel Biodegradable Thermoplastic Polyurethane Hydrogels for 3D Printing

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Introduction

3D printing, especially for the production of biodegradable biomedical implants, is severely limited by the availability of printable materials (1). This study investigates the characteristics of a series of biodegradable thermoplastic polyurethanes (TPUs) for application in 3D printing. These TPUs were prepared from poly(ε-caprolactone), and poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε-caprolactone) tri-blockcopolymers (PCL-*b*-PEG-*b*-PCL) and hexamethylene diisocyanate (HDI). By adjusting the composition of the TPUs, materials with varying hydrophilicity can be prepared.

Experimental Methods

Triblock copolymers with a targeted Mn of 20 kg/mol were prepared by ring opening polymerization of CL using PEG as an initiator. Triblock copolymers of different compositions were prepared (PCL_7-PEG_6-PCL_7, PCL_5-PEG_{10}-PCL_5, PCL_3-PEG_{14}-PCL_3) and reacted with HDI to form the TPUs (TPU-(PCL_x-PEG_y-PCL_x). The tensile properties of these TPUs were determined in the dry- and in the wet state using compression moulded films. 3D printing of these materials was done using an extrusion-based 3D printer (Biobot, 3D Systems).

Results and Discussion

Tensile testing showed that the TPUs exhibited excellent and tuneable mechanical properties (Table 1).

Table 1. Tensile properties of the different TPUs determined under dry and wet conditions.

	Water uptake (%)	E (MPa)	σ_{max} (MPa)	ε_{break} (%)
TPU-(PCL_3-PEG_{14}-PCL_3)	dry	100±15	6.1±0.4	1080±242
TPU-(PCL_5-PEG_{10}-PCL_5)	dry	176±16	8±2	766±282
TPU-(PCL_7-PEG_6-PCL_7)	dry	103±16	15±7	1566±326
TPU-PCL_{20}	dry	257±23	21±8	976±214
TPU-(PCL_3-PEG_{14}-PCL_3)	very high	fragile	fragile	fragile
TPU-(PCL_5-PEG_{10}-PCL_5)	534	7±2	4±1	147±41
TPU-(PCL_7-PEG_6-PCL_7)	122	52±10	17±2	1553±155
TPU-PCL_{20}	53	254±35	31±7	1349±243

These TPUs could readily be processed into porous scaffolding structures by extrusion-based 3D printing. Figure 1 shows such a hydrophilic structure prepared from the thermoplastic TPU-(PCL_5-PEG_{10}-PCL_5) hydrogel.

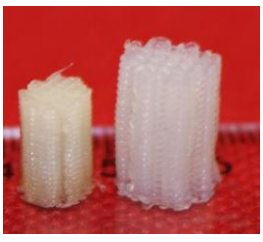

Figure 1. 3D printed TPU-(PCL_5-PEG_{10}-PCL_5) structure in the dry state (left) and in the wet state (right).

Conclusions

TPU hydrogels based on PCL-*b*-PEG-*b*-PCL triblock copolymers and HDI are promising materials for 3D printing applications.

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Polymeric Nano- and Micromaterials for Drug Targeting to Tumors

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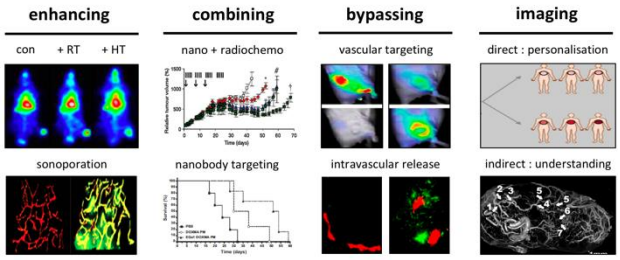
Summary

Nanomedicines are 1-100(0) nm-sized carrier materials designed to improve the biodistribution and target site accumulation of systemically administered (chemo-) therapeutic drugs. By delivering drug molecules more efficiently to pathological sites, and by preventing them from accumulating in healthy tissues, nanomedicines are able to improve the balance between efficacy and toxicity. In the last decade, several nanomedicines have been successfully translated to the clinic [1,2]. Mechanistically, nanomedicines generally rely on the Enhanced Permeability and Retention (EPR) effect for efficient tumor accumulation, which is mainly based on leaky blood vessels, and which is notoriously known to be highly variable, both in animal models and in patients. We are working on "smart" systems and strategies to address this heterogeneity, on polymeric nano- and micromaterials to enhance tumor-targeted drug delivery, and on pharmacological and physical combination therapies to improve tumor targeting. Together, these constructs and concepts help to improve the (pre-) clinical performance of tumor-targeted drug therapies [3,4,5]. In the present lecture, several of these constructs and concepts will be highlighted, including the use of polymeric microbubbles in combination with ultrasound to enhance tumor perfusion and vascular permeability, the use of polymeric micelles for imaging and targeting metastatic cancers, and the development of companion diagnostics and nanotheranostics for patient selection and (more) personalized nanomedicine therapy.

Schematic Figure



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Acknowledgments

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Tailor-made functionalized polymers for nanomedicine applications

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Introduction

Natural and synthetic polymers constitute the starting materials for the production of functionalized biomaterials able to produce nanoscaled smart delivery systems very promising in nanomedicine. In this context chemistry has enormously contributed to the development of smart materials with specific bio-properties or able to be responsive for biomedical applications; besides the cooperation among biology, chemistry, chemical engineering, medicine made possible the combination of materials and architectures in order to generate new effective therapeutic nanosystems.

Experimental Methods

The expertise of the LBP concerns the design, synthesis and characterization of new functionalized polymers starting on either natural or synthetic polyaminoacids and polysaccharides to produce nanostructured drug delivery systems and nanomedicine useful for the treatment of different pathologies using different kinds of drugs including genetic materials.

Results and Discussion

Polyaspartamide and polysaccharide derivatives have been produced to obtain nanosystems useful for different nanomedicine approach.

A overview of some representative examples of these research will be given[1-4].

Matryoshka formulations containing Ivacafotr loaded mucus and cell penetrating nanoparticles have been produced by using smart functionalized copolymers based on polyhydroxyethylaspartamide using Nano into micro (NiM) strategy; these systems showed to be useful for the restoring of the defect of CFTR in cystic fibrosis (CF) after pulmonary administration[1].

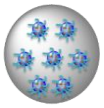

Figure 1. Nano into micro systems for the administration of drug in the CF.

NiM pulmonary dry powder formulations of tobramycin have been also successfully produced[2].

A new polymeric derivative with glyco-polypeptide architecture (PAA-VC) bearing L-arginine, vancomycin and colistin as side chains acting against multiple targets, able to give rise to a broad spectrum antimicrobial activity has been produced and tested[3].

Inulin-based nanodevices for the delivery of siRNA have been prepared and tested with particular reference to their chemical design and structure, biocompatibility, siRNA complexing ability, silencing ability[4].

Conclusions

Tailor-made functionalized polymers for specific nanomedicine applications including pulmonary drug administration in CF, antibacterical activity in multi drug resistance and siRNA delivery have been produced.

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Polymer-based nanomedicines as a tool for personalized tumor treatment

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Introduction

Recent years have witnessed an exponential growth of nanotechnology-based drug delivery systems, called also "nanomedicines". Various water-soluble polymers, polymeric micelles, liposomes, polymersomes and nanoparticles are explored to improve disease treatment. Among studied systems the significant position belongs to water-soluble synthetic copolymers based on *N*-2-hydroxypropylmethacrylamide (HPMA). The HPMA copolymers are well known as highly potent carriers of drugs or their combinations or as highly hydrophilic material suitable for coating of various nanocarriers (e.g. gene vectors, nanoparticles). They enable attachment of drugs by biodegradable spacers, thus the active drug can be released from the polymer carrier in stimuli-sensitive. Not only tailored drug activation, but also effective drug targeting to the treated tissue is the crucial step for successful treatment.

Results and Discussion

The presentation will focus on the impact of nanomedicine structure and physico-chemical characteristics on the biological behavior of nanomedicines in vitro and in vivo, mainly on their biodistribution, tumor accumulation, in vitro cytotoxicity and in vivo treatment efficacy. Various polymer carriers and their drug conjugates intended for passive or CD19/CD202/CD38-based active targeting delivery to aggressive lymphomas were studied, including simple linear HPMA copolymers, diblock copolymers or the more sophisticated biodegradable grafted, micellar and star high-molecular-weight polymer conjugates. In vivo imaging of HPMA-based polymer conjugates proved a substantial impact of the drug carrier structure, e.g. molecular weight, structure of the biodegradable spacer or molecular architecture, on body distribution of both, the polymer carrier and the drug.

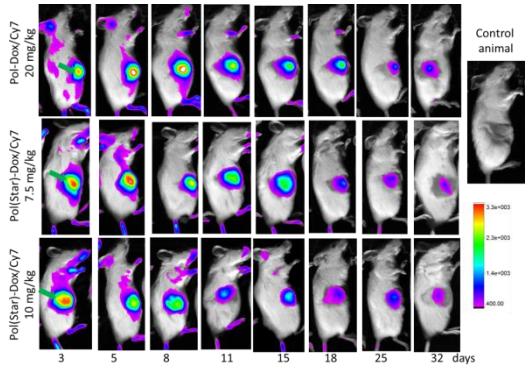

Figure 1. *In vivo* optical imaging. Serial *in vivo* optical imaging of RAJI tumour-bearing mice injected with various nanomedicines.

Obtained results showed a high potential and capability of nano-sized HPMA copolymer-drug conjugates for specific delivery of drugs and their combinations to aggressive lymphomas and thus for their efficient treatment. Even more, by the proper selection of targeting unit we can obtain highly efficient nanomedicines in the treatment of relapsed and highly resistant tumors.

Acknowledgments

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Reprogrammable Soft Shape-Memory Actuators with the Option of Degradability and Self-healing

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The classical shape-memory effect in polymers has so far been limited to its one way character. Upon triggering the shape-memory effect by heat, the material recovers its original shape. Cooling does not reverse this shape change. This limitation was overcome with the realization of shape-memory polymer actuators (SMPA), which can repetitively change their shape fully reversibly under stress-free conditions [1,2]. A conceptual novelty in this soft actuator class is the re-programmability of their shape changing geometry and the switching temperature.

Recently, non-continuously responding actuators based on crosslinked blends of poly(ε-caprolactone) and poly[ethylene-*co*-(vinyl acetate)] were introduced [3]. The stepwise movement, in which an actuation can be stopped without an additional external trigger or antagonist, represents a new level of complexity in the movement of soft actuators. Soft actuator materials are of technological significance for modern robotics. SMPA have been successfully tested for several hundred actuation cycles without any performance loss. A strategy to even prolongate the lifetime of a soft actuator after slight damage is the implementation of a self-healing capability [5].

Polydepsipeptides, alternating copolymers of an α-amino acid and a α-hydroxy acid, are an interesting group of degradable polymers. Depsipeptide containing block copolymer can be obtained by ring-opening or polyaddition of suitable oligomeric precorsors [6]. The incorporation of a polydepsipeptide segments in multiblock copolymers enables the combination of the advantageous degradation behavior of the depsipeptide segment with the shape-memory capability of multiblock copolymers [7]. The shape-memory effect can also be implemented and quantified for polymeric micro/nanowires. Pure oligodepsipeptides have been selected as a hydrophobic block in segmented polymers in order to achieve strong physical interactions stabilizing nanoparticles during their formation. These degradable block copolymers have shown great potential to combine transfection capability with low toxicity of the transfection agent [8].

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Polysaccharides for Imaging and Treatment of Cardiovascular Diseases

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Introduction

Atherothrombotic diseases remain the main cause of morbidity and mortality. There is a need for new approaches for early diagnosis and improved therapies for cardiovascular diseases. We have previously identified and patented a low molecular weight polysaccharide, fucoidan, as a powerful ligand of intravascular thrombi. Fucoidan refers to a type of polysaccharide mainly extracted from brown seaweed, which contains L-fucose and sulphate ester groups, able to mimetic Sialyl Lewis X, the endogenous saccharidic motif recognized by platelet P-selectin expressed in thrombi. Our aims were to i) prepare and characterize Fucoidan and several nano/microsystems coated with fucoidan to target the thrombus in preclinical models of ii) prepare a clinical grade Fucoidan for the demonstration in a clinical setting

Experimental Methods

Fucoidans from brown algae, containing low molecular weight polysaccharide species were prepared. The molecular weight, structure and composition of the purified fucoidan were determined by High Performance Size Exclusion Chromatography, Multi-Angle Laser Light Scattering, viscosimetry, differential Refractive Index, colorimetric assays, Fourier Transform InfraRed spectroscopy and Nuclear Magnetic Resonance. Fucoidan was directly complexed with ^{99m}Tc for SPECT imaging[1].

Several nano/microsystems containing Fucoidan were investigated: 1) We have designed ultrasmall superparamagnetic iron-oxide (USPIO) nanoparticles associated with fucoidan to visualize by MRI arterial thrombi[2]. 2) Microparticles of 2.5 µm were prepared by a water-in-oil emulsification combined with a cross-linking process of polysaccharides dextran, pullulan and fucoidan[3]. 3) Stable microcapsules (2-6 µm) made polysaccharide and functionalized with fucoidan were designed[4]. 4) Solid spherical nanoparticles (136±4 nm) containing fucoidan were synthesized and then also loaded with a clinical thrombolytic drug[5].

We have analysed *in vitro* the binding efficiency to recombinant P-selectin and to activated platelet aggregates under venous and arterial flows, and the efficiency in a mouse model of thrombosis by monitoring the platelet density with intravital microscopy, and in the intraluminal thrombus associated with progression of abdominal aortic aneurysms in rats.

Results and Discussion

The purification techniques for fucoidan have been optimized for industrial development of a pharmaceutical grade fucoidan. We obtained in 2015 from the French regulatory agency ANSM the label "raw material for pharmaceutical use".

Fucoidan with ^{99m}Tc (Radio-purity >95%) efficiently bind to P-selectin in several animal models of thrombus. *In vitro* experiments on human activated platelets, ex vivo flow chamber assays under arterial and venous shear stress conditions, and *in vivo* experiments on rat and mouse models evidenced targeting of fucoidan on P-selectin in thrombi. This effect has been observed for the fucoidan alone or on the surface of several nano/microsystems. In addition, polysaccharide based-nanoparticles targeted with fucoidan improved the thrombolysis efficiency of a loaded clinical drug (Actilyse) in thrombosis acute phase.

The Investigational Medicinal Product Dossier containing all regulatory safety data and product descriptions, the Investigator's Brochure and the clinical protocol were prepared. We obtained in January 2018 and March 2018 the agreements from French and Dutch medical agencies, respectively, to start the phase I clinical trials of the polysaccharide as a contrast agent for SPECT imaging of atherothrombosis. These clinical trails are expected to start in summer 2018 in Paris and Amsterdam

Conclusions

Fucoidan efficiently bind to P-selectin in several animal models of thrombus. Clinical grade fucoidan was prepared and clinical trials are expected in summer 2018 for molecular imaging of thrombus. Fucoidan-functionalized polymeric nano/microparticles were also developed with fucoidan acting as a targeting agent in several delivery systems. This could improve the prevention, diagnosis and treatment of atherosclerosis.

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Functional Degradable Polymers for Advanced Applications in Clinical Imaging

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Introduction

Medical devices and tissue engineering applications are largely relying on polymeric biomaterials that are intrinsically not visible under the clinical imaging modalities currently in use. In this contribution, we report on various advanced functional polymers, especially polyesters, that have been designed and synthesized to make them visible under scanner (X-ray) or medical resonance imaging (MRI).

Experimental Methods

Various strategies have been followed to yield polymers for clinical imaging. These include functional monomers synthesis and copolymerization (lactones, acrylates), direct polyester post-polymerization modification in solution or surface modification of polymer substrates. All these strategies have been applied to X-ray or MRI contrast agents including iodine, triiodobenzoic acid (TIB), gadolinium complexes (Gd-DTPA or Gd-DOTA) or superparamagnetic iron oxide nanoparticles (SPIONs). Imaging tests have been performed both *in vitro* and *in vivo* to evaluate the visibility, stability and perennity of the vizualisation.

Results and Discussion

In a first strategy, iodo-poly(ε-caprolactone) (PCL-I) and triiodobenzoate-PCL (PCL-TIB) have been successfully prepared via an anionic activation of PCL with LDA as a strong base; followed by nucleophilic substitution with I_2 or TIB. The resulting radiopaque copolymers have been used as additives to prepare implantable degradable biomaterials.[1] A good and stable visibility under micro-computed tomography analyses was confirmed in vivo in a rat model (Fig. 1a). In a second strategy, clickable polyester or poly(meth)acrylates have been synthesized via copolymerization of alkyne functional monomers followed by CuAAc reaction with azido-functional Gd ligands. These MRI-visible copolymers have been used as coatings for surgical meshes and demonstrated their high potential as T1 contrast agents *in vivo*.[2-4] Finally, in a last approach, polymer surfaces, including PLA electrospun fibers, PLA-Plu®-PLA threads or PP surgical meshes were efficiently modified with photoreactive Gd ligands (Fig. 1b) or photoreactive SPIONs. The absence of bulk properties modification as well as the ability of these functional surfaces to be imaged under T1 or T2 MRI modalities both *in vitro* and *in vivo* have been assessed.[5]

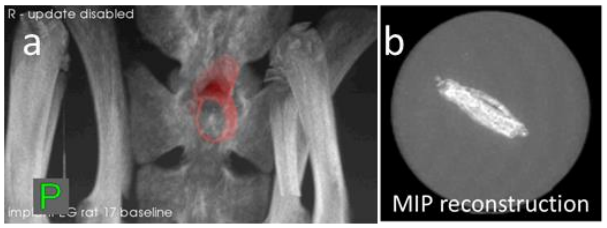

Figure 1. Example of radiopaque copolyester (in vivo, µCT) (a) and of MRI visible PLA-Plu®-PLA threads (b)

Conclusions

Through various chemical strategies, a family of polymers for clinical imaging of medical implants is proposed.

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Poster abstracts

Drug-releasing Biopolymeric Structures Manufactured via Stereolithography

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Introduction

Additive manufacturing, especially fused deposition modelling (FDM) and stereolithography (SLA), have become increasing popular within the biomedical field. Drugs have been incorporated into SLA resins and FDM filaments to prepare oral drug-delivery devices. However, to the best of our knowledge, the incorporation of a drug into a SLA-manufactured implant has not been previously investigated. SLA enables the preparation of predesigned structures with tailored drug release rates and profiles, which will open new possibilities to prepare bioactive implants.

Experimental Methods

PCL macromers were synthesized as described previously[1]. Cylindrical scaffolds with diamond pore architecture and porosities of 79.4, 70.4 and 59.3% were designed and built via SLA. The resin contained 10wt-% of model drug lidocaine and 90wt-% PCL macromer.

Thermogravimetric analysis (TGA) was used to determine the drug content in the printed structures, while differential scanning calorimetry (DSC) was used to evaluate the dissolution of the drug into the PCL network. Drug release was monitored in phosphate buffer solution (pH7.4) using UV/Vis-spectrophotometry.

Results and Discussion

Figure 1 shows the CAD model design with a porosity of 79.4% and its corresponding manufactured scaffold. The obtained porosities were: 75.4% (D80), 68.9% (D70) and 55.2% (D60). The surface area of scaffolds grew slightly with porosity. According to TGA, the amount of drug within the scaffold was 9.4wt-%.

The DSC confirmed dissolution of lidocaine into the PCL matrix, since no melting peak of lidocaine was observed. Figure 2 displays the drug release from different scaffolds, reaching the release of 52 to 68% over a period of 6 days.

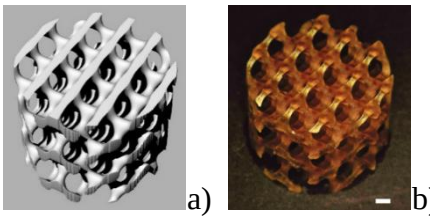

Figure 1. a) CAD model and, b) SLA-manufactured PCL structure. Scale bar is 1mm.

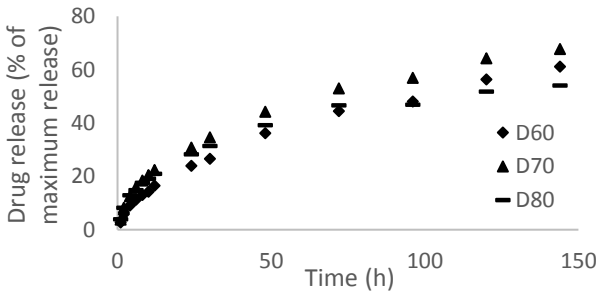

Figure 2. Drug release from PCL scaffolds having different porosities at 37°C (n=6).

Conclusions

Porous, drug-containing PCL scaffolds with a designed architecture were successfully manufactured by SLA. 52 to 68% of the drug was released over a period of 6 days.

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The authors would like to thank The Finnish Concordia Fund and KAUTEfoundation for providing financial support to this project.

Polycationic Particles Based on Polyethyleneimine and Poly(ethylene glycol) Functionalized Oligoester

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Introduction

Polycationic particles created by self-assembly are promising candidate materials for the transfection of DNA,[1] whereby the transfection efficiency is affected by the sizes of carrier systems.[2] Here we explored how size and surface charge of these particles can be controlled by variation of the molecular parameters, e.g. type of hydrophobic block segment in polycationic triblock copolymers as well as content of a neutral diblock copolymer as co assembly agent. To explore these effects, polycationic particles were designed based on triblock copolymers from polyethyleneimine (PEI) and oligo[(*ε*-caprolactone)-*co*-glycolide] (CG) and CG functionalized poly(ethylene glycol) (PEG-CG) diblock copolymers. Glycolide units were integrated randomly into the hydrophobic block segments in order to increase the amorphous character of the material. In this series, the oligoester block was composed of *ε*-caprolactone units.

Experimental Methods

Block copolymers and polycationic particles were synthesized according to the procedures described in reference.[3] The particle size, polydispersity index, and zeta potential were determined by Dynamic Light Scattering (DLS) using a ZetasizerNano (Malvern Instruments, Herrenberg, Germany).

Results and Discussion

Nano particles in the size from 35 to 98 nm with controllable positive surface charges were obtained when the type of block segment as well as content of diblock copolymer were varied. Particles based on CG were substantially smaller than those from particles without glycolide units. Furthermore, the presence of glycolide significantly reduced the polydispersity index. Here it was assumed, that the increased amorphous character as detected by scanning electron microscopy as result of the integration of glycolide would enhance the mobility of polymer chains and would support the self-assembly process. The surface charge increased when the content of the neutral diblock copolymer was reduced resulting in zeta potential values from 2.9 to 18.1 mV, whereby no specific relation between diblock content and particle size was found.

Conclusions

In this work structure – properties relationships of polyester-based particles created by self-assembly were explored. Here, the surface charge was controllable by the content of the diblock copolymers and the adjustment of particle sizes could be addressed by increasing the amorphous character of copolymers.

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Thermal Crosslinking of the Hydrophilic Polymers in their Melts

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Introduction

Every day, the number of patients that suffer from life threatening diseases that require organ transplantation increase [1]. In the U.S. alone, more than 116,000 patients need a lifesaving organ transplant and, on average, every 10 minutes one person is added to the waiting list [2]. Besides insufficient donor organs, organ transplantation bear other risks. The field of tissue engineering hopes to overcome these limitations. Hydrogels are important material class for tissue engineering and biofabrication due to the broad spectrum of accessible properties and have been extensively investigated for biomedical application (e.g. drug delivery systems, tissue engineering, organ transplantation etc.) [3, 4, 5]. Here we present a strategy to produce hydrogels by thermal crosslinking of non-functionalized hydrophilic polymers in their melts. Our approach has the advantages that it is solvent free, allows to crosslink functionalized as well as non-functionalized polymers and may be employed at different temperature.

Experimental Methods

For this purpose we are using polyperoxides, i.e. polymers bearing peroxide side chains, synthesized at the department of the Organic Chemistry, Ukraine [6]. Chloroform was purchased from Acros and dried over $CaCl_2$. Poly(ethylene glycol) (PEG) and poly(2-oxazoline)s with different molecular weight were obtained from Sigma-Aldrich. The schematic illustration of the crosslinking process is shown below, (Figure 1).

Results and Discussion

We investigated the dependence of the crosslinker on the polyperoxide and hydrophilic polymer concentration, temperature as well as curing time. While the crosslinker concentration markedly influenced the swelling degree, the curing time had a minor effect (Figure 2).

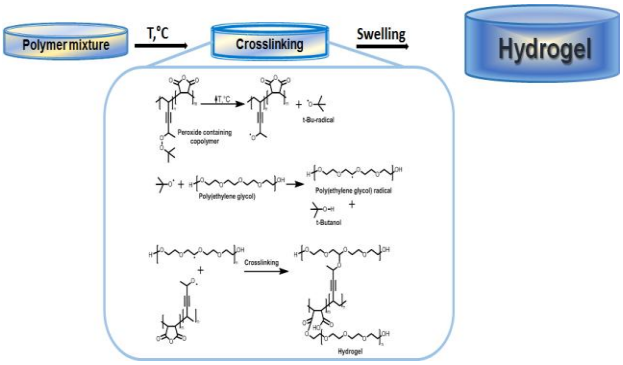

Figure 1. Schematic illustration on the thermal crosslinking hydrophilic polymers in their melts.

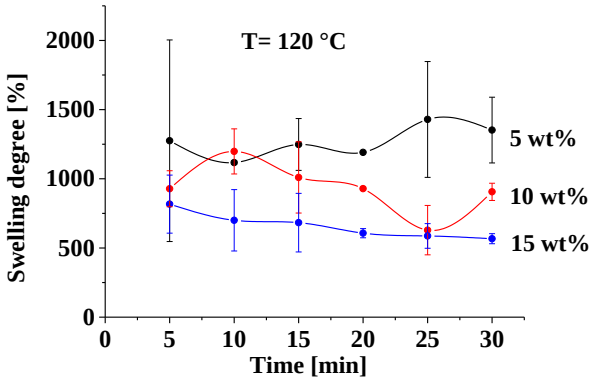

Figure 2. The swelling degree of the hydrogel film depends on the concentration of crosslinker and, to a lesser degree, on the heating time. The temperature of the process is 120 °C.

In the further work will be investigated crosslinking process during the shorter heating time and higher temperature as well as it influence on the hydrogel properties.

Conclusions

In summary we successfully crosslinked non-functional polymers using polyperoxides and could control the swelling degree of the resulting hydrogels.

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Acknowledgments

Solomiia Borova is grateful for a PhD scholarship by the Bayerisches Hochschulzentrum für Mittel-, Ost- und Südosteuropa.

Fabrication of Poly (trimethylene carbonate)/reduced graphene oxide-graft-poly (trimethylene carbonate) Electrically Conductive Scaffolds for Nerve Regeneration

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Introduction

Nerve regeneration remains a big challenge in spite of surgical intervention and entubulation [1]. Conductive, biocompatible and flexible electrospun scaffolds have been recognized as promising nerve substitutes to promote nerve regeneration due to their benefits of electrical stimulation and resemblance of the natural extracellular matrix [2]. Currently, reduced graphene oxide (rGO) material that is well-known for its high electrical conductivity has been incorporated in biocompatible polymers to manufacture conductive scaffolds in nerve tissue engineering. However, rGO/polymer composites tend to become inhomogeneous as the concentration of rGO increases. To overcome this problem, we replaced rGO with rGO-PTMC which was prepared by grafting trimethylene carbonate (TMC) oligomers onto rGO. The aim of this study was to engineer electrically conductive scaffolds for nerve regeneration via electrospinning of mixed solutions of PTMC and rGO-PTMC.

Experimental Methods

A dispersion of PTMC (3%, w/v) and rGO-PTMC was prepared by dispersing of PTMC and rGO-PTMC into a chloroform/dimethylformamide mixture solvent with vigorous magnetic stirring. Different ratios of rGO-PTMC to PTMC between 0~4 wt% were prepared. For electrospinning, the homogeneous dispersions were placed into a 5 mL standard syringe equipped with a 27G blunted stainless steel needle using a syringe pump at a rate of 1.0 mL/h with an applied voltage of 12 KV. The needle to collector distance was set at 15 cm. The electrospun fiber mesh was characterized by scanning electron microscopy (SEM), FTIR and TGA.

Results and Discussion

Surface morphologies of PTMC/rGO-PTMC scaffolds were examined by SEM. As shown in Figure 1, ultrafine electrospun scaffold was obtained for pure PTMC and the PTMC composites. The fiber morphology did not show apparent difference as the proportion of rGO-PTMC increased from 1.2% to 4.0%.

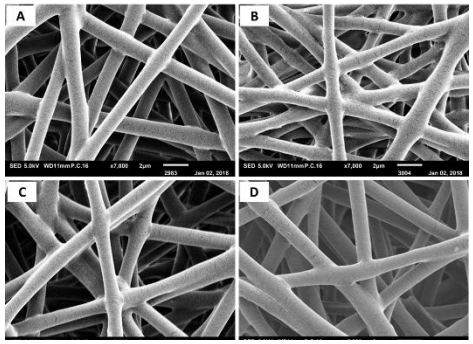

Figure 1. SEM images of rGO-PTMC/PTMC scaffolds with different proportion of rGO-PTMC. (A) 0%; (B)1.2%; (C)2.4%;(D)4.0%;

Conclusions

rGO-PTMC/PTMC scaffolds with ultrafine fiber morphologies were successfully fabricated by electrospinning.

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Acknowledgments

The authors appreciate Foryou Medical Company for kindly providing TMC monomer.

Synthesis of a new Biodegradable Copolymer for Intra-cellular Anti-cancer Agent Release

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Introduction

Nowadays, several drug delivery systems have been developed, in particular polymeric nano-objects, such as micelles or nanoparticles, based on amphiphilic copolymers.
Conventionally, these structures are composed of hydrophobic chains grafted along a hydrophilic backbone.
This project aims at synthesizing a new biocompatible and biodegradable graft copolymer with an original "reverse" structure composed of a hydrophobic backbone (polyester) and hydrophilic oligosaccharide side chains. Then, nano-systems with new versatile physico-chemical and biological properties will be prepared. [1]

Experimental Methods

Chitosan oligomers were synthesized by a depolymerization method described by Illy et al. [2] Propargyled poly-(ε-caprolactone) (PCL-yne) was prepared by an anionic activation process. [3] All other reagents were purchased from Sigma Aldrich and used as received.

Results and Discussion

First, 3-azidopropanal was prepared by a Michael addition reaction between acrolein and sodium azide. [4] Then, azido-chitosan was synthesized (Fig 1a) and was then grafted onto PCL-yne backbone by a CuAAC click chemistry reaction (Fig 1b). Azide substitution degree of azido-chitosan and Hydrophilic-Lipophilic Balance (HBL) of the copolymer were determined by ^{1}H-NMR analyses.

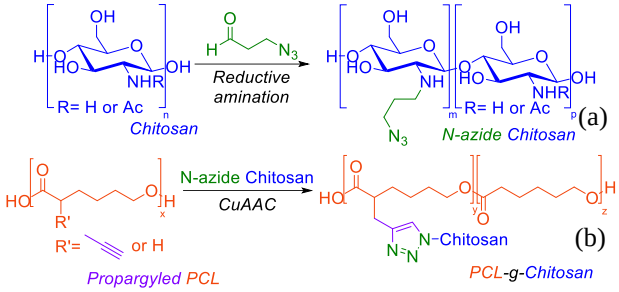

Figure 1. (a) azidation of chitosan, (b) CuAAC click chemistry reaction between PCL-yne and azido-chitosan.

Nano-objects were then prepared by a nanoprecipitation process. Nano-object size was determined by DLS analyses.

Conclusions

A new "reverse" copolymer structure was successfully synthesized. Drug loading of nano-objects based on this copolymer is currently under investigation: drug loading, encapsulation efficiency and drug release kinetics of these new polymeric nano-systems will be determined.

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Acknowledgments

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Additive Manufacturing of Bioactive Composites for Bone Grafting

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Introduction

In bone grafting or bone substitutes, utilization of synthetic biomaterials in composites with β-tricalcium phosphate (β-TCP) fillers show promising results due to their osteoconductive properties. Polymer composites including 60 wt-% β-TCP have demonstrated to have similar osteogenic activity as pure β-TCP [1]. Additive manufacturing enables the creation of patient specific bone regenerating implants. However, printing structures with such high TCP content is a challenge. In this study, high-resolution polymer based, resorbable and bioactive composite scaffolds for bone regeneration were successfully manufactured by stereolithography. These structures contained up to 51 wt-% β-TCP.

Experimental Methods

Poly(trimethylene carbonate) (PTMC) was synthetized by ring opening polymerization of trimethylene carbonate and further functionalized with methacrylic anhydride as previously described [2]. The PTMC macromer (M_n=9500g/mol) was dissolved in chloroform together with β-TCP (Sigma Aldrich) and the solution was precipitated in cold ethanol. After drying the composite was mixed with propylene carbonate, TPO-L (IGM resins) and Orasol orange (CIBA Speciality Chemicals) to create a printable resin. Designed structures with a gyroid pore architecture and an 83% porosity were built using stereolithography (SLA). Furthermore, networks were crosslinked for tensile testing.

Results and Discussion

Mechanical experiments of the crosslinked networks show a tensile modulus of 230 MPa for networks containing 52 wt-% β-TCP compared to 52 and 3MPa for networks containing 40 and 0 wt-% respectively. Designed porous structures from PTMC containing β-TCP were successfully built using SLA (Figure 1). Thermogravimetric analysis indicated β-TCP contents of up to 51 wt-% in the scaffolds. Work on similar structures containing lower amounts of a bone inducing component showed improved bone healing [3] indicating the great potential of the obtained structures.

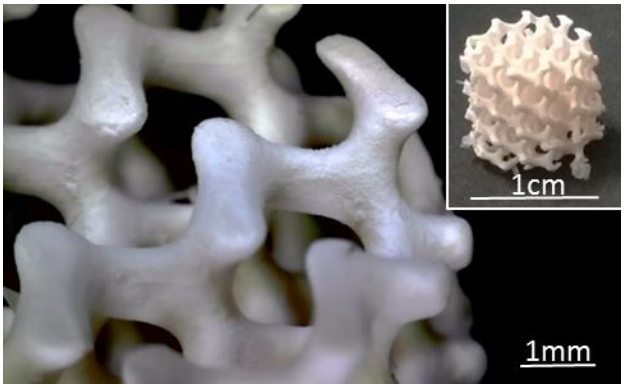

Figure 1. PTMC scaffold containing 51 wt-% β-TCP with a gyroid pore architecture.

Conclusions

The presented porous structures are to the best of our knowledge the highest TCP containing reported for PTMC composites manufactures by SLA. The composites show remarkable properties for use as resorbable bone grafting implants.

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Limited Shape-dependency of Phagocytosis for Polymer Micronetworks

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Introduction

The phagocytosis of particles by specialized cells such as macrophages or dendritic cells aims at removal of presumably pathogenic particulate matter from the body. This process is highly relevant in Pharmaceutics as it influences the delivery function of micro-/nanoparticulate drug carriers. In the last decade, the role of shape dependency of phagocytosis has been identified, where non-spherical ellipsoidal particles showed reduced engulfment [1]. One has to note that those mechanistic studies exploring the interaction of single cells and particles may not represent the conditions of multiple cell-particle contacts as present in vivo. It would therefore be of high interest to further explore the role of shape and size on phagocytosis at various cell-particle ratios using application relevant polymer particles generated from degradable polyester materials.

Experimental Methods

Polymer micronetworks based on oligo(ε-caprolactone) precursors [2] were prepared by emulsion based templating with subsequent crosslinking of methacrylate endgroups by UV irradiation (308 nm). Particles were characterized for size (Malvern Mastersizer M2000) and shape (SEM, Gemini Supra^{TM} 40 VP, Carl Zeiss NTS). Suspensions of micronetworks in polyvinyl alcohol solutions were casted, followed by drying to obtain films, which were stretched in a tensile tester to produce ellipsoidal particles. For uptake studies, RAW264.7 macrophages ($1 \cdot 10^5$/well) were cultivated in DMEM-medium with 50 µL MN suspensions in 96 well plates and analyzed at different time points by flow cytometry and confocal laser scanning microscopy.

Results and Discussion

Poly(ε-caprolactone)-based spherical polymer micronetworks with an average diameter of 6 and 10 µm were loaded with a fluorescent dye and transferred into different ellipsoidal shapes by a stretching method. Uptake studies using RAW264.7 macrophages illustrated that shape effects can only be found for certain conditions when varying particle sizes and shapes, cell-particle ratios (1:1, 1:2, 1:10, 1:50) and time points (1-24 h) under conditions of multiple cell-particle contacts.

Conclusions

Controlling shape effects on phagocytosis with application relevant polymers strictly requires considering further parameters besides shape, e.g. kinetic aspects of cell exposure or surface treatment to modulate particle recognition in different shapes.

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Degradable Double Hydrophilic Block Copolymers for the Formulation of Drug loaded Stimuli-Dependent Micelles

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Introduction

In the last two decades, double-hydrophilic block copolymers (DHBCs) consisting of two or more water-soluble blocks of different chemical nature have gained increasing attention. In DHBCs the first block (often based on poly (ethylene glycol), PEG) promotes solubilization in water, whereas the second block is responsive to an external stimulus or capable of interacting with another polymer or substrate.[1] The main objective of this work is the synthesis of novel DHBCs for biomedical applications including the formulation of micelles allowing the intracellular controlled release of drugs and biologics.[2,3]

Experimental Methods

A family of DHBCs based on PEG and functional PCL is obtained in three steps by combination of ring opening polymerization (ROP), anionic activation/nucleophilic substitution and thiol-yne photoaddition.

Results and Discussion

PEG_{2k}-*b*-$PCL_{1.5k}$ diblock copolymers are prepared by ring opening polymerization (ROP) between mono-methoxyPEG (MeO-PEG-OH) as initiator and ε-caprolactone. The PCL block is then functionalized with carboxylic, amine or hydroxyl groups in two steps: first propargylation is achieved by nucleophilic substitution; second the functional groups are introduced by thiol-yne photoaddition. In particular, focus is given to PEG_{2k}-*b*-$PCL_{1.5k}$(COOH). High substitution degree of 40% is obtained yielding a copolymer that forms pH-responsive micelles. The size (ca. 200 nm) of the nanoaggregates and their CAC (<0.9 mg/mL) decrease with pH varying from 7.4 to 5. The PEG-*b*-PCL(COOH) was used in the frame of doxorubicin and SiRNA encapsulation. For example, the encapsulation efficiency of the DOX by the DHBCs is higher (60%) than the encapsulation efficiency by the classical amphiphilic PEG-*b*-PCL (25%).

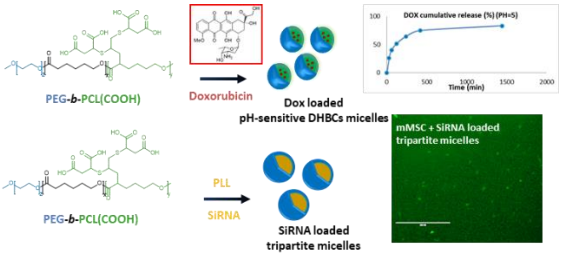

Figure1. Examples of application for the PEG_{2k}-b-$PCL_{1.5k}$(COOH) DHBCs.

Conclusion

Different degradable DHBCs have been synthetized via a simple methodology. They could demonstrate high potential for various biomedical applications including encapsulation of ionic API or of SiRNA.

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Oligopeptide–Polymer Conjugates for Targeted Imaging of Solid Tumors: Synthetic Strategies and Biological Evaluation

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Introduction

Specific oligopeptide–polymer conjugates can be used in variety of applications such as probes for targeted tumor cells imaging, delivery systems for anticancer therapy or as a theranostic tool which combine both strategies together. In this study, we focus on chemical synthesis of the oligopeptide–polymer conjugates targeted either to integrins coupled with tumor neovasculature ($\alpha_V\beta_3$ integrin), or epidermal growth factor receptor (EGFR), which is overexpressed on the surface of many types of tumor cells. Binding efficacy of fluorescently labeled oligopeptide-polymer conjugates was evaluated *in vitro*.

Experimental Methods

We prepared polymer conjugates based on the poly(*N*-(2-hydroxypropyl)methacrylamide) (pHPMA) copolymers labeled with fluorescent dye Cyanine 5.5 or Dyomics 633. GE7 (NPVVGYIGERPQYRDL), GE11 (YHWYGYTPQNVI) and RGD oligopeptides were attached to the polymer backbone aminolytically or by copper-free click chemistry. Binding efficacy of prepared conjugates was evaluated by flow cytometry (FACS) using epidermal growth factor receptor (EGFR) positive human cervical adenocarcinoma (HeLa) cells or $\alpha_V\beta_3$ integrin receptor positive human umbilical vein endothelial cells (HUVECs).

Results and Discussion

Prepared polymer conjugates were characterized using size exclusion chromatography (SEC) and high performance liquid chromatography (HPLC). Amount of fluorescent dye was determined spectrophotometrically and the content of peptides was evaluated using amino acid analysis. FACS analysis showed that GE11–polymer conjugate bound to EGFR on HeLa cells with higher affinity than GE7-polymer conjugate. Additionally, evaluation of linear RGD, fork-like PEG4- or PEG12-RGD, and cyclic RGD polymer conjugates demonstrated the highest binding efficacy to $\alpha_V\beta_3$ integrin receptor for the fork-like PEG12-RGD. This effective binding is based the most probably on the optimal distance of RGD's from polymer and simultaneously on the multivalency of fork-like bound oligopeptide, which altogether enabling to reach the recognition site inside the receptors [1].

Conclusions

Our results demonstrate that the GE11 and fork-like PEG12-RGD-pHPMA polymer conjugates provide efficient binding to specific cell receptors and thus might be used as a promising imaging tool.

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Support by the Ministry of Health of the Czech Republic (grant 16-28594A) and Czech Science Foundation (grant 16-04863S and grant 16-17207S) is gratefully acknowledged.

Phthalocyanine photosensitizer molecularly dispersed in polymeric nanocarriers

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Introduction

Chloroaluminium phthalocyanine (AlClPc) is a second generation photosensitizer under investigation for use in photodynamic cancer therapy.[1] Due to its geometry and lipophilic character, the dye aggregates in aqueous medium and loses its photosensitizer property.[2] This work presents the preparation, physico-chemical and photo-physical characterization of AlClPc in polymeric nanospheres (NS) from polyethylene glycol-*block*-poly(D,L-lactide) (PEG-PLA) capable of maintaining the dye molecularly dispersed in aqueous medium.

Experimental Methods

AlClPc loaded NS were prepared by nanoprecipitation of PEG-PLA. The UV-Vis absorption and steady-state fluorescence spectra of the NS suspensions were acquired in water. The NS size, size-distribution and fluorescence emission were determined after incubation in cell culture medium containing 10% fetal bovine serum (FBS) and following separation by asymmetric flow field flow fractionation (AF4 system Postnova AF2000, Germany) with UV, dual wavelength fluorescence, multi-angle laser light scattering (MALS) and dynamic light scattering (DLS) detection in series.

Results and Discussion

The UV-Vis (Fig 1A) and fluorescence spectra indicate similar photophysical properties of AlClPc within the NS as in acetonitrile, a good solvent for AlClPc, indicating that the dye is molecularly dispersed within the nanocarrier. Incubation in cell culture medium containing serum proteins the NS maintained their colloidal stability and size-range, without significant dye transfer to the proteins (Fig 1B).

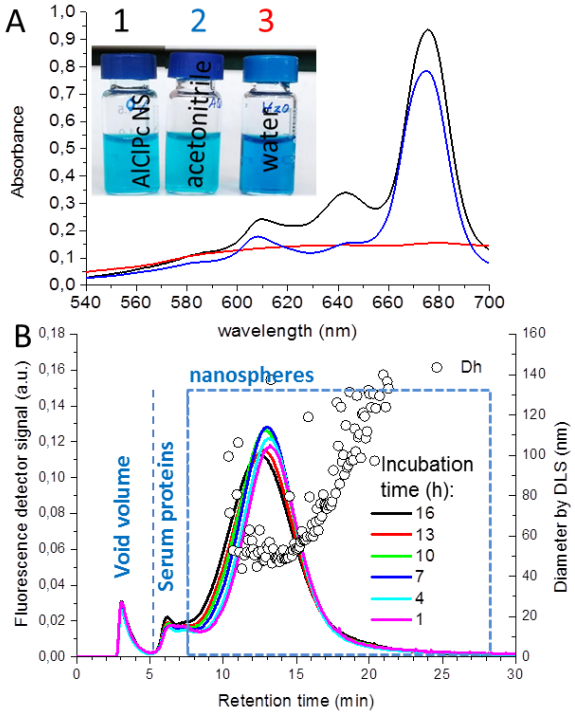

Figure 1. AlClPc absorption maximum (A). NS size and fluorescence emission by AF4 (B).

Conclusions

PEG-PLA NS prevent AlClPc aggregation in aqueous medium, providing a simple and promising photosensitizer formulation.

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Controlled Polyplex Release From a Thermosensitive Hydrogel

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Introduction

Nucleic acid-based drugs have emerged as a new class of therapeutics, as they can modulate the cellular expression levels of specific genes and their functional proteins. Especially in the development of novel cancer treatments, nucleic acid-based therapies have gained a lot of interest. However, the delivery of nucleic acids into the target cells is challenging, indicating the requirement of an advanced delivery system.[1] The aim of our research is to develop an easily injectable self-assembling hydrogel that is able to release nucleic acid (NA)-loaded polyplexes (Figure 1). The formation of an hydrogel provides a controlled and local release of the drug-containing nanoparticles.

Experimental Methods

We synthesized ABC triblock copolymers consisting of a thermosensitive (A), hydrophilic (B) and a NA-complexing (C) block via radical polymerization using a novel hetero-functional PEG macroinitiator.[2] *N*-isopropylacrylamide (NIPAM) and 2-(dimethylamino)ethyl methacrylate (DMAEMA) monomers were used for the A and C blocks, respectively. Polymers were characterized by ^{1}H-NMR, GPC and light scattering. Hydrogels were prepared by dissolving the polymers and plasmid DNA (pDNA) at 4 °C in HBS buffer, pH 7.4 overnight followed by incubating them at 37 °C.

Results and Discussion

ABC triblock copolymers were successfully synthesized, with two different thermosensitive block lengths (Table 1). Upon the addition of pDNA, the ABC polymers were able to self-assemble into polyplex nanostructures of approximately 180 nm and with a PDI of 0.24. Characterization of the polyplexes by light scattering experiments, ^{1}H-NMR studies and gel electrophoresis assays showed no significant effect of changes in temperature below and above the cloud point (CP) of the polymer, indicating that the polyplex structure remained stable at 37 °C. In addition, the DNA-loaded polyplexes were able to deliver their cargo into cancer cells *in vitro*. In the next step, the polyplexes were mixed with ABA polymers to prepare a thermosensitive hydrogel at 37 °C and polyplex release was measured by pDNA quantification. Polyplexes prepared with an ABC polymer containing a longer thermosensitive block (25 kDa) showed a slower release from the hydrogel compared to when a polymer with a shorter thermosensitive block (14 kDa) was used (Figure 2). This indicates that besides dissolution of the hydrogel also thermosensitive interactions play a role in the kinetics of polyplex release.

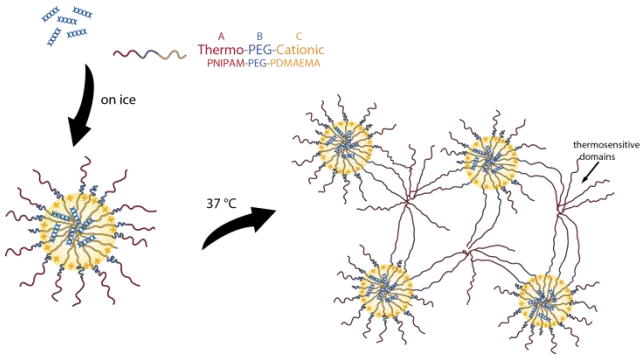

Figure 1. Schematic representation of polyplex and hydrogel formation.

Table 1. Characteristics of ABC triblock copolymers consisting of NIPAM (A), PEG (B) and DMAEMA (C).

	$A_{14}B_5C_{48}$	$A_{25}B_5C_{43}$
M_n A (kDa)[a]	14	25
M_n B (kDa)[a]	5	5
M_n C (kDa)[a]	48	43
Total M_n (kDa)[a]	67	73
Total M_n (kDa)[b]	41	50
PDI[b]	2.0	2.0
CP[c] (°C)	34	34

[a]Determined by ^{1}H-NMR. [b]Determined by GPC. [c]Determined by light scattering at 550 nm.

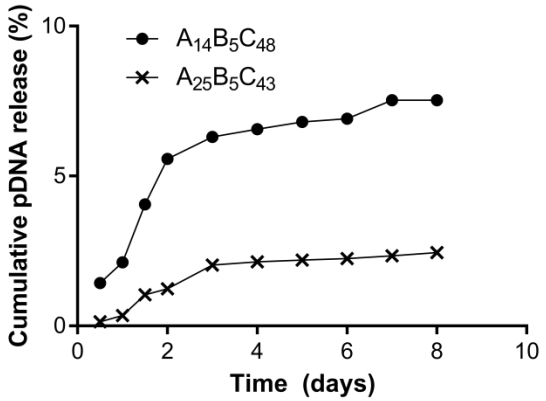

Figure 2. Release profile of polyplexes from a thermosensitive hydrogel.

Conclusions

Our findings show that the polyplex release from the thermosensitive hydrogel was dependent on thermosensitive interactions and the dissolution rate of the hydrogel system, which can be tailored via polymer block composition. We believe that these data show the potential of such polyplex-releasing hydrogel systems for the controlled and local delivery of nucleic acids via an easily injectable formulation.

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Synthetic strategy to thiol functionalized polyesters and their biomedical applications

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Introduction

The degradable aliphatic polyester family has gained interest for applicability in biomedical field for manufacturing of resorbable tissue engineering scaffolds and drug release systems.[1] However, the lack of functional groups along the polymeric chains limits their applications. We have addressed this issue by designing and synthesizing a lactide-type monomer featuring a latent thiol group, the TrtS-LA.[2] We reasoned that, due to its chemical versatility, the presence of thiol functionality could allow post-polymerization modifications, thus, tuning the physical and chemical properties and opening the way to new potential applications for this class of materials.

Experimental Methods

The synthesis of TrtS-LA monomer and catalyst was previously reported,[2] as well as procedures for polymerization,[2] scaffolds[2] and nanoparticles[3] preparation and characterization.

Results and Discussion

The TrtS-LA has been copolymerized with several monomers, and by using different catalysts, achieving a good control over the chain growth.[2,3]

The latent thiol groups were modified into pyridyl disulphide functionalities and the polymer was subsequently used to fabricate editable porous scaffolds. A cysteine-terminated RGD peptide was covalently attached to the porous scaffolds by a disulphide-exchange reaction in aqueous solution. Thus, scaffolds for tissue engineering with enhanced biological response could be fabricated.[2]

Furthermore, we have prepared redox-responsive PEG-PLA based nanoparticles by reaction of pyridyl disulphide functionalized polyesters with a telechelic PEG having thiol groups at both ends. In water solution, they assembled in flower-like particles, with size in the range 167 – 300 nm, which is suitable for drug delivery. The redox responsiveness to glutathione was also ascertained, showing that the prepared nanoparticles could be used as drug carrier with controlled and targeted release.[3]

Conclusions

TrtS-LA revealed to be a useful building block for the preparation of functionalized aliphatic copolyesters, whose potential applications in biomedical field have been assessed. Such polymers can be used both for editable scaffolds fabrication and for the preparation of redox-responsive nanoparticles.

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Absorbable and biocompatible architected materials dedicated to soft-tissue reconstruction

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Introduction

Synthetic materials commonly used in soft-tissue engineering have low deformability and are generally not bioresorbable. Beyond those intrinsic properties, their structuration plays a key role. The development of efficient biomimetic scaffolds requires the control of three-dimensional structures having interconnected pores to promote host-tissue reconstruction [1]. In this work, we evaluate the impact of various patterns for the electrospinning of architected degradable scaffolds and the promotion of cellular growth.

Experimental Methods

PLA-PEG-PLA, PLA-Pluronic®F127-PLA triblock copolymers well-defined structures were synthesized via ring-opening polymerization for 5 days at 130°C. Polymer solutions in DCM/DMF (70/30 v/v) were prepared and electrospun with an applied voltage around 15kV. The analyses of morphology and orientation of the micro-fibers were performed by SEM (ultra 55 SEM FEG, Zeiss, Jena, Germany). Mechanical measurements were carried out using ARES-G2 rheometer (TA Instruments, New Castle, DE, USA). Degradation tests samples were conducted in PBS (pH 7.4) at a constant temperature. Primary cells, NHDF fibroblasts were used to evaluate the *in vitro* compatibility of the scaffolds.

Results and Discussion

The triblock copolymer PLA-PEG-PLA having low molecular weight (100 kg.mol^{-1}) showed great potential to control architecture and pore size of our scaffolds compare to triblock copolymers with higher molecular weight (200 kg.mol^{-1}) and PLA-Pluronic®F127-PLA. 3D micro-structured scaffolds were elaborated thanks to micro-fibers with bimodal distribution of diameters promoting attractive domains over the mats of the collector [2]. Mechanical properties of our scaffolds could be modulated by varying the nature of unit mesh (Figure 1).

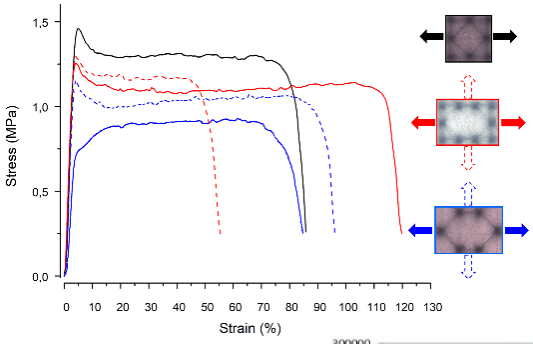

Figure 1: Mechanical properties of architected scaffolds based on PLA-PEG-PLA triblock copolymer

A preliminary *in vitro* study with L929 fibroblasts was conducted on different architected scaffolds based on PCL. It showed that the architecture may influence cellular growth (Figure 2).

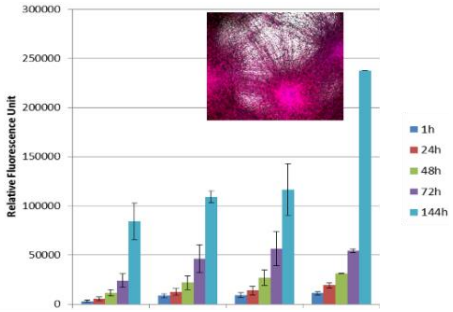

Figure 2: L929 cell proliferation on architected scaffolds

Conclusions

By modulating mechanical properties of our scaffolds, a better fit could be found with the host-tissues. A further study on the *in-situ* photo-crosslinked fiber will be investigated to increase intrinsic scaffolds properties.

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Implementing and Quantifying the Shape-Memory Effect of Single Polymeric Micro/Nanowires with an Atomic Force Microscope

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Introduction

Recent work has successfully translated shape-memory effect (SME) technology to micro/nanoscale polymeric objects where complex behaviors, such as multiple shape change and reversible shape-memory effects, have made shape memory materials desirable for a range of biomedical applications. However, the implementation of SME in polymeric micro or nano-objects currently relies on the application of indirect macroscopic manipulation techniques, e.g. stretchable molds or phantoms, to ensembles of small objects.[1] Here we introduce an AFM-based method capable of the controlled manipulation and SME quantification of individual electrospun micro and nano-objects in analogy to macroscopic thermomechanical test procedures.

Experimental Methods

An atomic force microscope was utilized to address individual electro-spun poly(ether urethane) (PEU) micro- or nanowires freely suspended between two micropillars on a micro-structured silicon substrate. Hereby, the AFM cantilever was used to deform and fix single wires to a temporarily bended shape.

Results and Discussion

Programming strains of 10 ± 1% or 21 ± 1% were realized for micro- and nanowires. An almost complete restoration of the original free suspended shape during heating confirmed the excellent shape-memory performance of the PEU wires. The thorough thermomechanical characterization enabled by this method yielded apparent recovery stresses of $\sigma_{max,\ app}$ = 1.2 ± 0.1 and 33.3 ± 0.1 MPa for a single microwire and nanowire respectively.

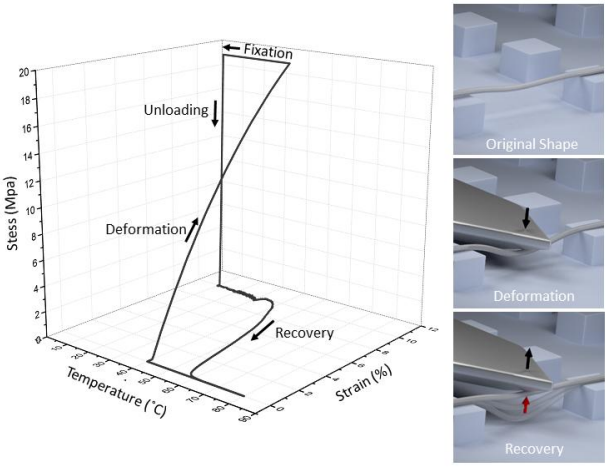

Figure 1. AFM derived stress-temperature-strain curves for an individual microwire. Schematic showing the deformation and quantification of the shape recovery of a polymeric microwire.

Conclusions

This work provides a platform for the translation of polymeric materials into useful nano/micro technologies by demonstrating comprehensive experimental micro/nanoscale characterization of mechanical behavior in analogy to cyclic, thermomechanical procedures used for characterization of macroscopic polymer specimens.

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Synthesis and Characterization of rGO-graft-poly(trimethylene carbonate) for Nerve Regeneration

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Introduction

Nerve regeneration in the case of long-gap peripheral nerve damage remains a challenge. Chemically converted graphene, especially highly reduced graphene oxide (rGO), has drawn a lot of attention due to its physical properties such as extremely high electrical conductivity. In our previous study [1], rGO/poly-(trimethylene carbonate) (PTMC) composites showed electrical conductivity and facilitated the growth of neuronal cells. However, at relatively high rGO concentrations, rGO/ PTMC composites tended to become inhomogeneous. The aim of this study was to solve this problem by synthesizing rGO-graft-PTMC, by using an rGO initiator for the polymerization of TMC.

Experimental Methods

Graphene oxide (GO) and rGO were obtained by a modified Hummer's method [1]. The method to prepare rGO initiator was followed from Gao's work [2]. Azido ethanol was reacted with pre-dispersed rGO in N-methylpyrrolidon at 160^{o}C under nitrogen atmosphere. The ring-opening polymerization of TMC monomer was carried out in toluene with rGO initiator at 110 ^{o}C under argon atmosphere, with stannous octoate as catalyst. The rGO-graft-PTMC was obtained by repeated suspending and centrifugation in dichloromethane and characterized by FTIR and TGA.

Results and Discussion

As shown in Figure 1, –OH groups (2900-3700 cm^{-1}) of GO were removed during reduction of GO with hydrazine monohydrate. The FTIR spectrum of rGO-graft-PTMC shows C-O (1230 cm^{-1}), C=O (1700 cm^{-1}) and C-H (2900 cm^{-1}) peaks due to PTMC on the surface of the graphene. From the content of rGO initiator as determined by TGA and the weight of the obtained rGO-graft-PTMC, the molecular weight of the PTMC was calculated. This amounted to 1000 g/mol.

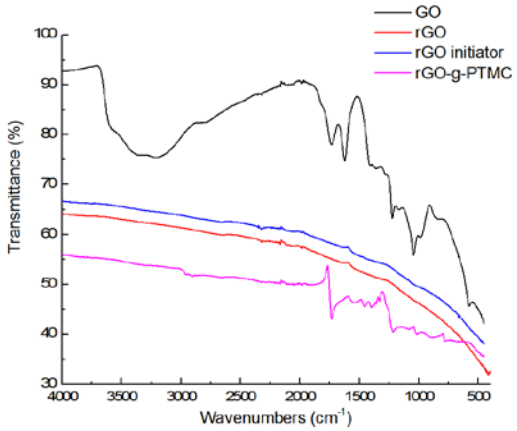

Figure 1. FTIR of GO, rGO, rGO initiator and rGO-graft-PTMC.

Conclusions

rGO-graft-PTMC was successfully synthesized by ring-opening polymerization of TMC using an rGO initiator.

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Investigation of Structure Property Relationship of Poly(2-oxazoline)s & Poly(2-oxazine)s based Structural Analogues for Highly Hydrophobic Anti-cancerous Drugs.

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Introduction

According to an estimate, 90% of all compounds in today's drug delivery pipelines are poorly water soluble [1]. There is an intense need and severe demand for the tailor made excipients, which not only increase the water solubility and sustained the biological activity, but also render the system biocompatible.

Motivated by the extraordinary high drug loading and high specificity of poly(2-methyl-2-oxazoline)-block-poly(2-butyl-2-oxazoline)-block-poly(2-methyl-2-oxazoline) (A-**pBuOx**-A) based micelles for **paclitaxel** [2,3] and A-poly(2-propyl-2-oxaine)-A (A-**pPrOzi**-A) for **curcumin** [4] we decided to explore the solubilizing capacity of the these structural analogues including two others i.e A-poly(2-butyl-2-oxaine)-A (A-**pBuOzi**-A) and A-poly(2-Pentyl-2-oxazoline)-A (A-**pPentylOx**-A) with a set of hydrophobic anti-cancerous drugs. The main objective of this study is not only to increase the solubility of hydrophobic molecules but also to investigate the structure property relationship between various drugs and 4 structural analogues for the further design of efficient excipients.

Experimental Methods

ABA triblock copolymers were synthesized by live cationic ring opening chain polymerization. Micelles were prepared by thin film hydration method with various polymer to drug ratio. Drug loading was determined by HPLC [4]. DLS was used for size analysis. Candidate drugs are **lapatinib, crizotinib, panobinostat, vorinostat, erlotinib, mitotane & bexarotene.**

Results and Discussion

A-**pBuOzi**-A have shown a very high drug loading of 53 wt%. when **lapatinib & paclitaxel** were co-formulated. A-**pBuOzi**-A was also found to be best solublizer for **Mitotane** as compared to other analogues and formulation (10:6) were stable up-to 30 days. Formulations were also stable upon redispersion in water, PBS and 40g/L bovine serum albumin. Currently we are extending our studies to bigger library of hydrophobic drugs to get insights into the structure property relationship.

Conclusions

POx and **POzi** based amphiphiles have shown higher solubilizing capacity for difficult to formulate drugs where all other polymers rarely exceed 20 wt% drug loading. Design of selective and highly specific carriers can help us to fulfill the high demand of excipients for such drugs.

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Advanced tracking into the intracellular localization of polymer therapeutics using fluorescence lifetime imaging microscopy and fluorescent pattern analysis

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Introduction

The *in vitro* evaluation of newly developed polymer therapeutics with bound drugs or diagnostic agents brings a better understanding of the nanocarrier characteristics. Moreover, it enables prediction of the behavior of the polymer therapeutics during *in vivo* application. Combination of microscopy and advanced fluorescence detection methods, e.g., fluorescence lifetime imaging microscopy (FLIM), is able to describe the properties of various polymeric nano-systems in various cell compartments and contributed to a complex view of drug or diagnostic agent intracellular transport. In present study, we employed FLIM in combination with computation method called pattern matching to characterized changes in the intracellular localization of cancerostatics – doxorubicin (DOX) or pirarubicin (THP) released from various types of polymer conjugates during the incubation with cells.

Experimental Methods

We prepared polymer conjugates based on the poly(*N*-(2-hydroxypropyl)methacrylamide) (pHPMA) or poly(2-ethyl-2-oxazoline) (PEtOx) copolymers carrying DOX or THP bound via hydrolytically cleavable spacer. The intracellular localization of released drugs was evaluated using an IX83 confocal laser scanning microscope (Olympus, Japan) equipped with a FLIM/FLCS upgrade kit (PicoQuant GmbH, Germany). The SymPhoTime64, 2.1 software was used for data acquisition, and analysis.

Results and Discussion

The FLIM image analysis was able to track the intracellular fate of the DOX released from pHPMA-DOX or PEtOX-DOX polymer conjugates after incubation with human breast carcinoma cells (MCF7). The different cellular localization of DOX was characterized by two fluorescence lifetimes (t1,t2). PHPMA-DOX shows a more pronounced localization of t1 in nuclei, similarly as free DOX, compared to PEtOx-DOX, which can be attributed to its higher cellular uptake and DOX nuclear localization.

A pattern matching analysis was used for the searching of specific shapes of fluorescence decay curves of defined FLIM patterns, which was in our case free DOX or THP. Using pattern matching we were able to monitor the differences in the localization of the drugs released from the polymer backbone in the human colorectal carcinoma cell lines (DLD1) incubated with pHPMA-DOX or pHPMA-THP. For pHPMA-DOX, no pattern component was localized in the nucleus, only in the cytoplasm. For pHPMA-THP pattern components were present in both the nucleus and the cytoplasm, which documented faster uptake of THP.

Conclusions FLIM and fluorescent pattern analysis are ready to open new horizons in determination of detailed information about the behavior of cancerostatics or polymer carriers inside the cells.

Acknowledgments

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Hydrolytic stability of aliphatic poly(carbonate-urea-urethane)s

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Introduction

Poly(carbonate-urethane)s (PCUs) exhibit improved resistance to hydrolytic degradation and *in vivo* stress cracking compared to poly(ester-urethane)s. In addition, a degradation of PCUs lead to lower inflammation of the surrounding tissues.[1] Therefore, PCUs are promising implant materials and are considered for devices such as the artificial heart or spine implants.[2]

Here the hydrolytic stability of different poly(carbonate-urethane–urea)s (PCUUs) is studied under variation of the precursors. We hypothesize that the lengths of hydrocarbon chain between the carbonate linkages (6, 9, 10 and 12 methylene units) in the precursors could influence the morphology, crystallinity, as well as hydrolytic stability of resultant PCUUs.

Experimental

Investigated PCUUs were synthesized by the moisture-cure method, utilizing isophorone diisocyanate and oligo(alkylene carbonate) diols as precursors.[3] The changes of the sample weight, as well as thermal and mechanical properties after immersion in a buffer solution (PBS, pH = 7.4) for up to 20 weeks at 37 °C were monitored. All of the analyses (FTIR, DSC, DMTA, tensile measurements, and WAXS) were performed as described in reference [3].

Results and Discussion

The weight of the samples did not change significantly after 20 weeks in PBS (water uptake up to 0.9 ± 0.2 wt% and weight loss up to 0.1 ± 0.1 wt%) for all investigated PCUUs. An increase of the melting point of PCUUs was observed and was the most pronounced for samples with dodecamethylene carbonate units (increase from 45 to 65 °C). Moreover, it was shown that the degree of crystallinity (DOC) increased from 16 ± 1% up to 21 ± 1% in case of samples containing 10 or 12 methylene units between carbonate linkages, whereas in case of those containing 6 or 9 methylene groups DOC decreased from 12 ± 1% to 8 ± 1%. Also, in case of latter two, a significant decrease of Young modulus from 43 to 35 MPa and from 18 to 10 MPa respectively, was observed. Nevertheless, in case of all samples the tensile strength was in the range of 30-40 MPa and elongation at break around 700-800% after 20 weeks in buffer at 37 °C.

Conclusions

Performed investigations confirm good resistance to hydrolysis of PCUUs. Observed minor changes in the crystallinity as well as thermal and mechanical properties depended on the number of methylene units between the carbonate linkages in the soft segment of PCUU.

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Comparative study of PCL shape-memory networks with Diels-Alder or Alder-ene adducts.

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Introduction

Shape-memory polymers (SMPs) are remarkable stimuli-responsive materials able to switch from one stable macroscopic shape to another one, which can find application as smart medical devices. For this purpose, poly(ε-caprolactone) (PCL) networks are widely studied because PCL is biocompatible, degradable and has good mechanical properties. The integration of reversible bonds in these networks allows (re)processing and recycling of the SMP material. In this work, different reversible reactions, i.e. Diel-Alder (DA) addition of TAD-anthracene or maleimide-Anthracene and Alder-ene (AE) addition of TAD-indole, were used for the synthesis of reprocessable SMPs.

Experimental Methods

Bis-TAD and star-shaped PCL end-capped by maleimide, anthracene or indole were synthesized as reported elsewhere[1,2]. They were melt blended (Table 1) at 120°C in a mini-extruder in equimolar ratio followed by thermal curing at 65°C for blend 1. The cross-linking density of the resulting networks was determined by swelling experiments, their crystallinity by DSC and SMP properties by DMA.

Results and Discussion

Blends	Mass of the first component (g)	Mass of the second component (g)
1	1.91(PCL-4ANTH)	2.49 (PCL- 4MAL)
2	4.00 (PCL-4ANTH)	0.30 (Bis-TAD)
3	4.00 (PCL-6IND)	0.15 (Bis-TAD)

Table 1: Composition of the different melt-blends.

The 3 blends reached a similar swelling rate (around 1000%) typical of a highly cross-linked network[1]. Remarkably, it is reached directly after extrusion for blends 2 and 3 thanks to the fast DA and AE additions while a post-curing of 24h at 65°C is required for blend 1. Expectedly, all the three networks exhibited a similar crystallinity degree above 35%, accounting for the good fixity of these SMPs. If recovery ratios higher than 99% were measured for the three samples, a creep effect was observed for the blend 3 upon reaching the temporary shape originating from the stress-sensitive AE adducts able to break upon deformation at 90°C. Finally, the reprocessing of these networks at 120°C was achieved only for blends 2 and 3 thanks to their fast retro-reaction.

Conclusions

Various DA or AE reactions were investigated to introduce reversible bonds in PCL networks leading all to SMPs with high fixity and recovery. Among them, the anthracene-TAD equilibrium combines (i) fast addition to build the network, (ii) high stress stability at 90°C avoiding creep phenomena during processing of the temporary shape, (iii) high reversibility above 120°C offering efficient modification of the permanent shape and material (re)processing and recycling.

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Design of a multimodal hyaluronic acid based hydrogel system to target inflammation in lipopolysaccharides treated mixed glia cells

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Introduction

Targeting inflammation in spinal cord injury (SCI) is a very challenging regenerative task.[1] Hyaluronic acid (HA) in its high molecular weight form has been known to elicit an anti-inflammatory response and is a promising candidate in this context.[2-5] Herein we report the synthesis and characterization of a high mol. wt. HA conjugates with reactive oxygen species (ROS) responsive functional groups; thiol (-SH) and dopamine (-Dop). We envisage that hydrogels of these conjugates can be multimodal systems to target inflammation which will be evaluated in lipopolysaccharides (LPS) treated mixed glia cells (MGCs) from rat spinal cord.

Experimental Methods

Medium and high mol. wt. forms of HA (500 KDa and 1MDa, Lifecore) were functionalized with -SH, -Dop and -SH-Dop via EDC/NHS chemistry to obtain six different potential conjugates for testing. The free thiol and disulfide bonds in these conjugates were estimated by Ellmans and Thannhauser assay and by measuring the absorbance maxima @280 nm in case of dopamine. Variables; buffer, pH and purification procedures were evaluated to obtain the polymer scaffolds of desired functionalities. NMR studies were carried out to estimate their purity and correlated the degree of substitution obtained from the assays. MGCs from rat spinal cord were used for metabolic activity studies.

Results and Discussion

A high degree of substitution -SH (43%) and -Dop (11%) was obtained only for reactions in MES compared to PBS buffer (Fig 1A&B). Reversible and irreversible hydrogelation was demonstrated at physiological pH with TCEP and PEG acrylate (Fig 1C). Co-incubation of hydrogels (10, 50 and 100 µg) with MGCs revealed no change in their metabolic activity up to 3d (Fig 1D).

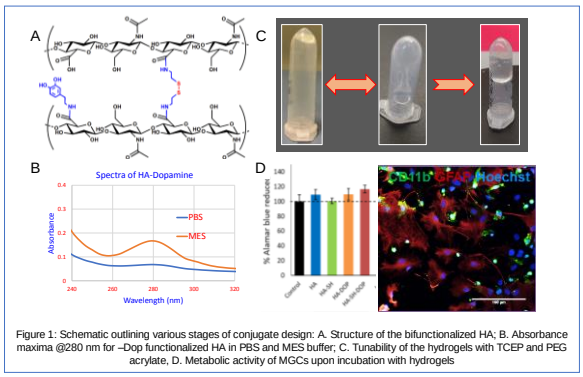

Figure 1: Schematic outlining various stages of conjugate design: A. Structure of the bifunctionalized HA; B. Absorbance maxima @280 nm for –Dop functionalized HA in PBS and MES buffer; C. Tunability of the hydrogels with TCEP and PEG acrylate, D. Metabolic activity of MGCs upon incubation with hydrogels

Conclusions

We have synthesized -SH and -Dop conjugated hydrogels of HA and evaluated their cytotoxicity in MGC cultures. Testing of their multimodal anti-inflammatory response towards LPS treated MGCs is currently underway.

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Synthesis of PCL-pHPMA graft copolymers for efficient tumor drug delivery

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Introduction

Drug delivery systems based on polymer carriers offer many advantages in tumor therapy, e.g. enhanced drug accumulation in tumor tissue, increased drug efficiency, reduced drug toxicity. Previously, we prepared various PCL-graft-poly(ethyleneglycol) systems with miscellaneous loaded drugs.[1] Here, novel amphiphilic graft copolymer poly(εcaprolactone)-*graft*-poly[*N*-(2-hydroxypropyl) methacrylamide-*co*-*N*-(6-(methacryloylamino)hexanoyl)hydrazine] (PCL-pHPMA) and its conjugate with anticancer drug, doxorubicin, bound via pH-sensitive hydrazone bond were synthesized and characterized.

Experimental Methods

PCL-propargyl was synthesized according to literature.[1] Semitelechic pHPMA-N_3 was synthesized by controlled radical RAFT polymerization using propargyl-containing chain transfer agent. PCL-graft-pHPMA was prepared using "click" reaction catalyzed by copper.

Results and Discussion

Amphiphilic graft PCL-pHPMA copolymer was formed by biodegradable hydrophobic PCL and hydrophilic biocompatible HPMA-based copolymer enabling drug attachment via a pH-sensitive spacer. The copolymer was prepared by the reaction of propargyl groups along the PCL chain with the pHPMA end azide groups in the presence of copper. The hydrazide groups were deprotected in distilled water at 100°C and used for the attachment of anticancer agent doxorubicin via pH-sensitive hydrazone bond. The deprotection reaction or drug attachment did not significantly influence the copolymer distribution of molecular weights (*Đ*). By simple dissolution PCL-pHPMA formed in aqueous solutions micelles with hydrodynamic diameter D_h = 75 nm.

The characteristics of graft copolymer and its precursors are given in Table 1. The degradation rate of biodegradable PCL-pHPMA is currently being tested.

Table 1. PCL, pHPMA and PCL-pHPMA characteristics

	M_n (g/mol)	*Đ*	D_h (nm)
pHPMA-N3	14 500	1.05	4
PCL-propargyl	14 300	2.10	-
PCL-pHPMA	67 200	1.99	75

Conclusions

Novel micelle-forming graft copolymer PCL-pHPMA represents flexible drug delivery system which can serve as an anti-cancer drug carrier with the active molecules attached via a pH-sensitive spacer and with biodegradable PCL core.

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Microgels of a silylated hydrogel as a multimodal system for drug co-encapsulation

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Introduction

Silylated hydrogels represents a new family of building blocks able to give chemical cross-linking, based on the hydrolysis/condensation reactions of alkoxysilane groups -$Si(OR)_3$ into stable siloxane Si-O-Si covalent links. They revealed a huge potential for biocompatible and injectable gels due to their ability to cross link in soft conditions of temperature and pH. Moreover a the wide range of hydrogels could be silylated, but also biomolecules such as peptides, proteins, ligands or fluorescent probes allowing to expect many applications from drug delivery to biomaterials. [1, 2]

The aim of this work was to (i) demonstrate the ability to produce microgels using silylated HPMC by the way of the Sol–Gel reaction of condensation of the -SiOR functions, (ii) to functionalize them, with a model function, using the same Sol-Gel reaction in a one-pot process (FMGs), and (iii) to generate biphasic microgels (BPMGs) containing lipophilic compartments intended to formulate poorly soluble drugs. Model drugs were used in the present work, Nile Red (NR) as lipophilic and silylated Fluorescein (Si-Fluor) as chemically linked function. [8]

Experimental Methods

The microgels FMGs and BPMGs were prepared by a water in oil templating (or oil in water in oil, respectively) process where Si-HPMC solution 3 wt.% was neutralized with HEPES at pH 3.5. The Si-FITC ethanolic solution was added to the Si-HPMC phase. This water solution was dispersed into the sesame oil continuous phase at a ratio of 1/10 v:v. The temperature was maintained lower than 10°C during the emulsification step. Then, the temperature was increased to 45°C and maintained for 10 min to induce the cross linking by condensation of the siloxanes functions.

Results and Discussion

Figure 1 summarizes the principle of formation of the functionalized microgels with the silylated fluorescein (FMGs) and the biphasic microgels (BPMGs), thanks to the critical physico-chemical parameters involved in the process. It was based on the sol-gel reaction and a templating emulsification process.

The pH and the temperature are the main parameters that control (i) the chemical cross-linking reaction, leading to the formation of Si-O-Si bonds and (ii) shaping and size distribution of the microgels. The Si-HPMC network could be functionalized with a model molecule, such as Si-FITC in FMGs using a water in oil emulsion templating process. Oil compartments for lipophilic molecules encapsulation, were trapped in the BPMGs with an oil in water in emulsion method.

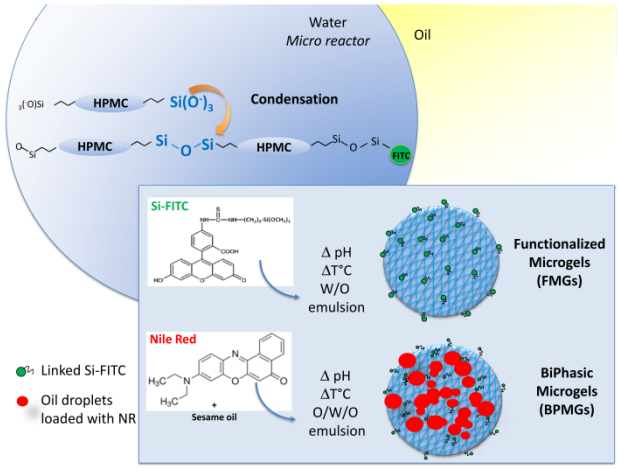

Figure 1. Principle of the production process of FMGs and BPMGs.

Figure 2 showed the morphologic and textural properties the BPMGs with a size distribution centered from 60 to 70 µm, an homogeneous spatial distribution of the oily compartments and the presence of a cellulosic network in the dried microgels.

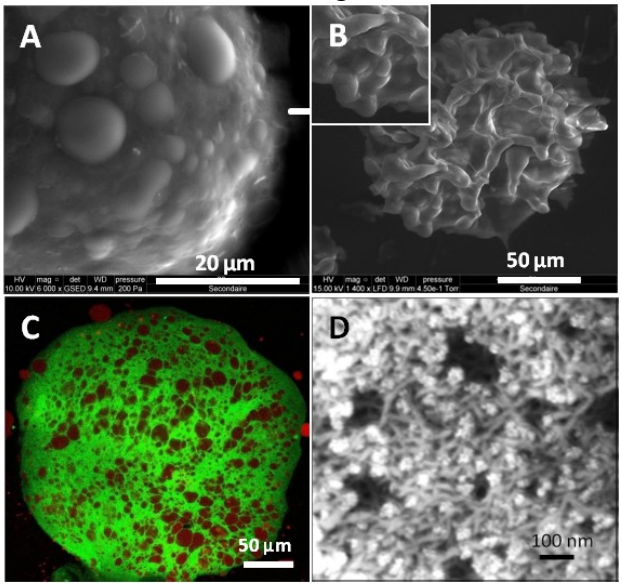

Figure 2. SEM observations of (A) hydrated and (B) freeze-dried BPMGs, their internal phase separation (C) between the hydrophilic and the lipophilic phases by LSCM, and the topography (D) of the Si-HPMC network of dried BPMGs determined by AFM.

Nile Red, used as a fluorescent probe and lipophilic drug, was directly loaded in the primary O/W emulsion up to saturation in the sesame oil, with a maximum of 25.0 mg/mL. Si-FITC was used as a covalently linked fluorescent probe and a model function. It was possible to graft up to 1.12 mg/g of Si-HPMC (or 70 mmole/mole) that could be relevant with a biological activity.

Conclusions

The preparation and the functionalization of microgels made of Si-HPMC were obtained by the unique condensation reaction of alkoxysilane functions in physiological conditions of temperature and pH. An emulsion templating process was applied using only the interfacial properties of HPMC without the use of any surfactant. The size distributions of the microgels are relevant with injections in implant sites for intra-articular, sub-cutaneous. This original approach of combining sol-gel chemistry for chemical cross linking and functionalization of hydrogels in a one-pot process, for drugs and biomolecules microencapsulation open up a promising way for new drug delivery systems. Work are now in process to improve the drug controlled release and the process of production to improve the size and morphologic control.

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Highly Stable RNA Uptake by Dense Cationic Polymer Brushes for the Design of Cytocompatible, Serum-Stable siRNA Delivery Vectors

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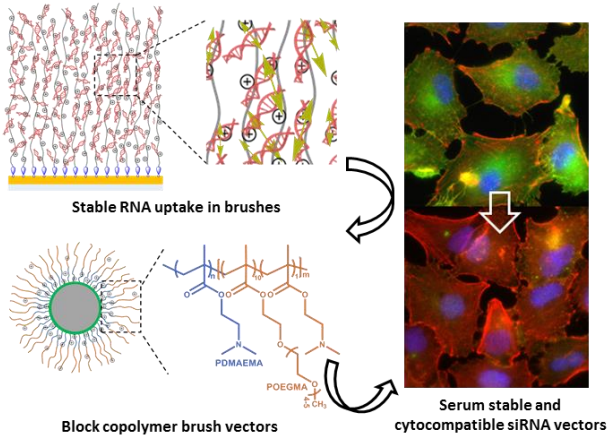
INTRODUCTION

The high density of polymer brushes confers to these coatings unique physico-chemical properties, in particular for the regulation of biomolecular interaction and the design of highly selective coatings for biosensors and protein patterning.[1] Here we show that high density poly(dimethylaminoethyl methacrylate, PDMAEMA) cationic polymer brushes enable the stable uptake of high levels of oligonucleotides. This is proposed to result from the high degree of crowding and associated increase in entropic driving force for the binding of polyelectrolytes such as nucleic acid molecules. We further demonstrate the ease with which such coatings allow the design of highly structured nanomaterials for siRNA delivery using block copolymer-brush based nanoparticles that allow the protection of oligonucleotides by a protein resistant outer block. In particular these nanomaterials display a high serum stability and low cytotoxicity whilst retaining excellent knock down efficiencies. Polymer brush-based nanomaterials therefore appear particularly attractive for the rational design of a new generation of high performance theranostics and RNA delivery probes.



Scheme 1. Design of dense cationic polymer brushes vector for Cytocompatible, Serum-Stable siRNA Delivery.[2]

METHODS and DISCUSSION

A series of DNA from 10 base pair to plasmid DNA and also siRNA were chosen to interact with PDMAEMA brush with 100 % and 10 % brush density. SPR results showed the binding of smaller genes strongly depends on brush density that dense brush can bind significantly more than spaced brush, whilst for larger genes, binding amount on both dense and spaced brush are similar but much lower than smaller genes. It may indicate that dense polymer brush coated nanoparticles might be more suitable for delivery smaller sized RNA such as siRNA or microRNA other than larger DNA (e.g. plasmid DNA). Based on the results above, we designed a dense block copolymer brush coated silica nanoparticle (SiO_2-block copolymer) vector for siRNA delivery, in which the first layer was pure PDMAEMA brush to encapsulate siRNA and the second block, poly(oligo(ethylene glycol) methyl ether methacrylate, POEGMA) brush increased the protein resistance and cell viability of the vector. SiRNA transfection with epidermal cells showed SiO_2-block copolymer have comparable knocking down efficiency with lipofectamine and increased the cell viability at the meantime.

In addition, to track the fate of particle/siRNA complexes during delivery and transfection process, we developed a method combining layer-by-layer self-assembly with ATRP. It allows us to label PDMAEMA brush decorated silica particles with fluorescent tags without changing any chemical or structural properties of the brush-particles and to study simultaneously the fate of brush-particles and oligonucleotides in situ during cell transfection experiments.

CONCLUSION

In summary, cationic polymer brush coated biomaterials could be a useful tool to understand gene-polymer interaction by controlled varying brush architecture, chemistry and bioactivity, which will provide important information for designing non-viral gene delivery vector.

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Photo-crosslinked Gelatin/PEG Hybrid Hydrogels for Tissue Engineering Applications

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Introduction

Hydrogels of natural polymers generally show good biological properties whereas the mechanical properties are poor. In contrast, hybrid hydrogels consisting of natural and synthetic polymers may show good biological as well as mechanical properties. The aim of this work was to prepare tough hydrogels consisting of gelatin and poly(ethylene glycol) (PEG) for tissue engineering applications. Both gelatin and PEG were functionalized by reaction with methacrylic anhydride. Mixtures of the methacrylated polymers were photo-crosslinked, after which the tensile properties of the hybrid hydrogels were investigated.

Experimental Methods

Gelatin (50-100 kg/mol) and PEG (4 kg/mol) were functionalized by reaction with methacrylic anhydride. The degrees of functionalization of Gelatin-methacrylate (Gel-MA) and PEG-dimethacrylate (PEG-dMA) were 37% and 99%, respectively[1]. Gel-MA and PEG-dMA were dissolved at concentrations of 20% (w/v) in de-ionized water at 50 ℃. Irgacure 2959 (0.05% (w/v)) was used as photo-initiator. Solutions with different ratios of Gel-MA:PEG-dMA were photo-crosslinked by irradiation for 30 min at 365 nm. The mechanical properties of the Gelatin-PEG hydrogels were investigated in the wet state by tensile testing according to ASTM D 638.

Results and Discussion

Stress-strain curves of Gelatin, PEG and Gelatin-PEG hybrid hydrogels are shown in Figure 1. By increasing the amount of PEG in the hybrid hydrogels, both the stiffness and maximum tensile stress increased. The 25%:75% Gelatin:PEG hybrid hydrogel showed the highest toughness (area under the curve) of 10.40 N/mm^2.

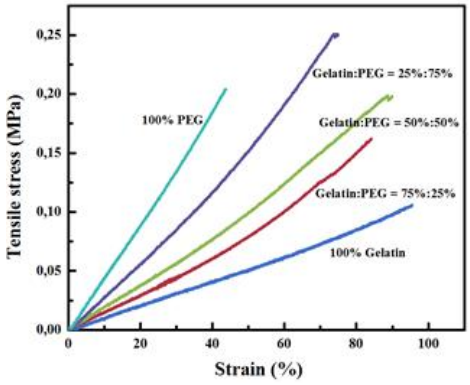

Figure 1. Stress-strain curves of Gelatin, PEG and Gelatin-PEG hybrid hydrogels. The toughness (area under the curve) of the different specimens was 3.99, 10.40, 7.06, 4.91, 5.18 N/mm^2 (curves from left to right).

Conclusions

Compared to a 100% Gelatin hydrogel, 25%:75% and 50%:50% Gelatin:PEG hybrid hydrogels have a higher toughness.

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Acknowledgments

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pH Changes of Aqueous Media Caused by Degradation of Suture Materials

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Introduction

The hydrolytic degradation process of suture materials *in vitro* is typically assessed by following the changes in tensile strength, sample mass or molecular weight of the polymers with degradation time. In this context, it has been shown that the pH level as well as the type of degradation medium (i.e. body fluids) influences the degradation behavior.[1,2] As the pH-level of an aqueous environment might be affected, in this study the initial pH-level changes of different degradation fluids exposed to poly(*p*-dioxanone) (PPDO) or poly(*ε*-caprolactone-*co*-glycolide) (PCG) are explored.

Experimental Methods

The monofil, sterile suture materials PDSII® (PPDO) and Monocryl® (PCG) (from Johnson & Johnson Medical GmbH, Ethicon Germany, Norderstedt) were incubated (0.2 g/L, minimum weight of 15 mg) in different degradation media (water, MEM medium with and without FBS serum, and PBS buffer solution) at 37 °C up to 48 hours while shaking (Certomat IS, Sartorius). The pH was measured (microelectrode InLab Micro, Mettler Toledo) at selected time points. In a second experiment, the initial degradation medium was removed after 30 minutes of incubation, then fresh medium was added and the pH change was recorded over time.

Results and Discussion

Kinetic studies showed a decrease of the pH for PPDO and PCG in water reaching a constant pH value within 3 hours, showing the main pH decrease in the first 30 minutes, attributed to the release of acidic products to the degradation medium. In contrast to water, the pH of the other degradation media was not affected or only slightly decreased (ΔpH < 0.4), regulated by the buffer capacity of the media. In the experiments with exchanging the water after 30 minutes, the overall pH change was less pronounced. The first contact to the degradation medium led to a pH decrease from 7.0 to 4.5 for PPDO and pH reduction from 7.0 to 5.0 for PCG, while much lower pH decreases of 0.4 (PPDO) and 0.2 (PCG) were found after the exchange of the degradation medium, attributed to the dissolution of degradation products absorbed at the suture surface rather than the formation of new degradation products.

Conclusions

Surprisingly, a more pronounced initial reduction in the pH of water as degradation liquid was caused by the polyetherester PPDO when compared to change in pH for the copolyester PCG. As an increased formation of new degradation products can be expected for PCG, the observed higher change in pH for PPDO might be explained by already existing degradation products, which diffuse into the degradation medium.

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Acknowledgments

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Drug specificity, synergism and antagonism in ultra-high drug loaded polymer micelles

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Introduction

According to estimates, more than 40% of all NCEs (new chemical entities) developed in the pharmaceutical industry are practically insoluble in water, illustrating the urgent demand for excipients, which increase the water solubility of such hydrophobic drugs to make them available to patients.

Experimental Methods

Drug-loaded micelles were thoroughly characterized using dynamic light scattering (DLS), differential scanning calorimetry (DSC), x-ray diffraction (XRD) and cryo-TEM measurements. Biological activity was evaluated with 3 different cell lines.

Results and Discussion

Motivated by the extraordinary high drug loadings of poly(2-oxazoline)s (POx) based micelles for paclitaxel (PTX) of more than 45 wt.%[1], this study investigates structure–property relationships of pseudo-polypeptide based amphiphiles on the solubilization for the non-water soluble drugs curcumin (CUR) and PTX. Besides an extremely high loading capacity for CUR of more than 50 wt%, we also observed strong drug specificities caused by minor changes in the polymer architecture.[2] The IC_{50} values of nano- or DMSO- formulated CUR were similar, showing that CUR was fully bioactive when incorporated into polymer micelles.[3] Compared to conventional 2D cell cultures, we observed an increase in resistance of MDA-MB-231 cancer cells in 3D tissue models (Figure 1).

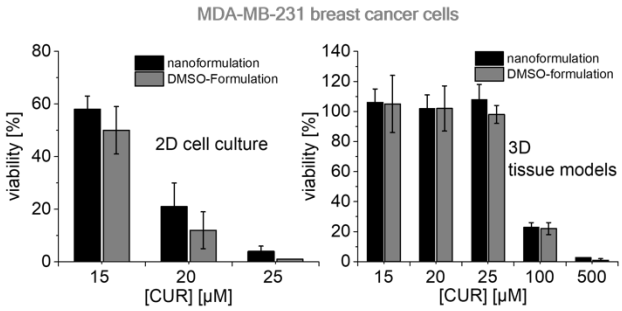

Figure 1. Cytotoxicity of nano-formulated CUR and DMSO/CUR in 2D-cell culture and 3D-tissue models of MDA-MB-231 cancer cells.[3]

Conclusions

As stability in complex biological media was higher for nano-formulated CUR compared to DMSO/CUR, the presented ultra-high drug-loaded nanoformulation might allow, for the first time, high-dose *in vivo* therapy using parenteral CUR administration necessary for effective therapeutic high-dose intervention.

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Acknowledgments

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Direct synthesis of peptide-modified silicone

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Introduction

Biocompatible and presenting great mechanical properties, silicone or polydimethylsiloxane (PDMS) is one of the most used polymer for the preparation of implantable medical devices and soft prosthesis.[1] As most synthetic polymers used in healthcare, PDMS is bio-inert. It has to be functionalised to display specific properties. The main strategy stays the post-functionalization of the polymer by a biologically-relevant molecule.[2] We proposed a new approach, based on the assembly of different blocs containing the desired functions to get a customizable multifunctional polymer.[3,4] We are working specifically on derivatives of silicones functionalised by bioactive peptides, drugs or fluorophores.

Experimental Methods

PDMS oils are prepared by polymerization of dichlorodimethylsilane (DCDMS) monomers with 1 to 10 mol% ratio of either dichloromethylvinylsilane (DCMSi-vinyl) or dichloromethylsilane (DCMSi-H) monomers, the two later being used for further reticulation. Hybrid silylated biomolecular blocs ($RSiMeCl_2$, with R being a peptide or any other molecule of interest) were added in the polymerization mixture in 0.1 or 0.01 mol%. Polymerization was performed using sodium dodecyl sulfate ([SDS]= 16.4 mM) as a surfactant in water at 60°C during 16h. The hydrosilylation was operated on a 50/50 ratio of two polymer chains, one containing the monomer functionalised by Si-H and the other by Si-vinyl, with a Karstedt's catalyst.

Results and Discussion

Materials obtained from oils containing 2.5 mol% ratio of silane or vinyl groups were found to display the best mechanical properties. An antibacterial peptide and a derivative of fluorescein were introduced during polymerization to get fluorescent or peptide-containing silicone oils. Antibacterial assays prove that the antibacterial activity is maintained on the resulting material after hydrosilylation.

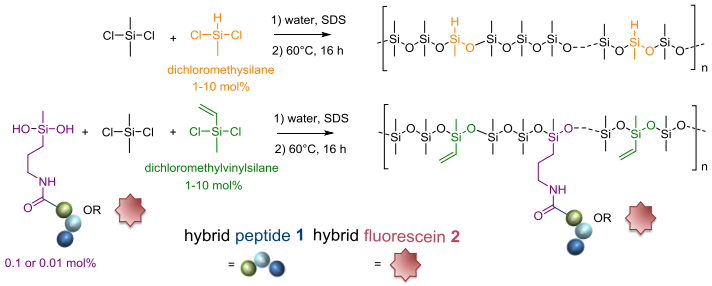

Figure 1. Copolymerization of DCDMS monomers with functional hybrid monomers.

Conclusions

This strategy enable the incorporation of a wide range of biomolecules into PDMS backbone, as long as they can be modified by a dichloromethylsilane moiety. It paves the way to many applications relying on multifunctional biomaterials. Indeed, any block can be combined in any ratio in a bottom-up way to get first, linear bioorganic PDMS oils then, hybrid bioorganic silicone materials.

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Tailoring the Thermo-Mechanical Properties of Degradable Copolyetherester Urethanes Comprising Oligo(*p*-dioxanone) and Oligo(*ε*-caprolactone) segments

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Introduction

Biodegradable shape-memory polymers are a promising class of multifunctional materials for realizing of active self-anchoring implants. [1,2]. One example are multiblock copolymers containing crystallizable, hydrolytically degradable oligo(*p*-dioxanone) (oDO) hard and oligo(*ε*-caprolactone) (oCL) switching domains [2,3]. The thermo-mechanical stability of such materials over a wide temperature range is a crucial aspect in the context of encoding the information about the desired shape change during the programming process and activation of the shape-memory effect within the body. Besides the number averaged molecular weight (M_n) of such phase-segregated copolyetherester urethanes, the overall crystallinity as well as the partial crystallinity of hard and switching domains represent a relevant contribution to the materials´ thermo-mechanical stability. In this study the influence of different oDO to oCL ratios on the multiblock copolymers´ thermo-mechanical properties and their nanostructure were investigated.

Experimental

Copolyetherester urethanes were synthesized by reaction of oligo(*p*-dioxanone)diol (5 kg·mol^{-1}) and an equal weight content mixture of three oligo(*ε*-caprolactone)diols with 3, 4 and 8 kg·mol^{-1} with *L*-lysine diisocyanate (LDI) in dimethylcarbonate. DSC, DMTA and tensile experiments at different temperatures were applied for examining the thermo-mechanical properties, while the crystalline nanostructure was analyzed by WAXS.

Results and Discussion

GPC results of the prepared copolyetherester urethanes revealed an increase in M_n from 13 to 130 kg·mol^{-1} with increasing the oDO content from 20 to 80 wt%. The partial crystallinities of oDO and oCL provided by DSC analysis were in good correlation with the respective content of the segments in the copolymer. The overall crystallinity determined by WAXS decreased from 35±1 to 24±1% with increasing oDO fraction. The dynamic modulus versus temperature curves obtained by DMTA showed a two-step decay around 50 °C for systems with ≥40 wt% oDO, while lower oDO contents caused material's failure once the oCL domains were melted. The mechanical properties of the copolymers at room temperature were almost similar having Young's moduli around 300 MPa, while at 60 °C the mechanical properties were solei governed by the oDO crystallinity with Young's moduli between 1 and 90 MPa.

Conclusions

Copolyetherester urethanes comprising an oDO crystallinity >15% exhibited a pronounced mechanical stability up to temperatures of 90 °C. In contrast an oDO crystallinity below 15% resulted in mechanical failure above the oCL melting transition.

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Acknowledgments

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Sustained delivery of siRNA/mesoporous silica nanoparticle (siRNA/MSN) complexes from nanofiber scaffolds for long-term gene silencing

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Introduction

A low toxicity and efficient delivery system is needed to deliver small interfering RNAs (siRNA) in vitro and in vivo. [1] The use of mesoporous silica nanoparticles (MSN) is becoming increasingly common due to its biocompatibility, tunable pore size and customizable properties. [2] However, bolus delivery of siRNA/MSN complexes remains suboptimal, especially when a sustained and long-term administration is required. A promising approach to attain sustained delivery of siRNA/MSN complexes is to incorporate them into scaffolds. As compared to bolus delivery, gene delivery from a scaffold offers more controllable and localized transfection.[3] This method not only minimizes systemic side effects but also enhances gene transfection efficiency.[4,5] However, to date, scaffold-mediated delivery of siRNA/MSN complexes has not been reported.

Experimental Methods

Here, we utilized electrospun scaffolds for sustained delivery of siRNA/MSN through surface adsorption and nanofiber encapsulation. As a proof-of-concept, we targeted collagen type I expression to modulate fibrous capsule formation *in vitro* and *in vivo*.

Results and Discussion

Surface adsorption of siRNA/MSN provided sustained availability of siRNA for at least 30 days in vitro. As compared to conventional bolus delivery, such scaffold-mediated transfection provided more effective gene silencing ($p < 0.05$). On the contrary, a longer sustained release was attained (at least 5 months) when siRNA/MSN complexes were encapsulated within the electrospun fibers. *In vivo* subcutaneous implantation and biodistribution analysis of these scaffolds

revealed that siRNA remained localized up to ~290 mm from the implants. Finally, a fibrous capsule reduction of ~45.8 % was observed after 4 weeks in vivo as compared to negative scrambled siRNA treatment. Taken together, these results demonstrate the efficacy of scaffold-mediated sustained delivery of siRNA/MSN for long-term non-viral gene silencing applications.

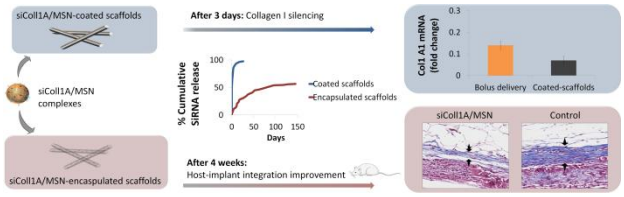

Figure 1. *In vitro* and *in vivo* potential of siRNA/MSN-coated scaffolds and siRNA/MSN-encapsulated scaffolds to reduce fibrous capsule formation

Conclusions

The biofunctionality of the scaffolds were further demonstrated in vitro and in vivo by targeting COLL1A1 as the model protein to decrease fibrous capsule formation to improve host-implant integration. Since MSN are also ideal for the delivery of low molecular weight drugs, this platform may also extend to future dual or sequential drug/gene delivery applications for directing cell fate and tissue regeneration.

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Noncontinuously Responding Polymeric Actuators

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Introduction

Current polymeric actuators move because of their continuous response to the input signals of the controlling unit and cannot be interrupted without stopping or eliminating the input trigger. Here, we present a new type of non-continuously responding actuators based on cross-linked blends of two crystallizable polymers, which are capable of pausing their movements in a defined manner upon continuous cyclic heating and cooling [1].

Experimental Methods

The crosslinked blend networks (cPCL-PEVA) were prepared by mixing poly[ethylene-*co*-(vinyl acetate)] (PEVA) and poly(*ε*-caprolactone) (PCL) with dicumyl peroxide in a twin-screw extruder and subsequent compression molding at 200 °C. Thermal properties of the actuator materials were investigated by DSC measurements. Atomic force microscopy (AFM) and scanning electron microscopy (SEM) were used to investigate the morphology of cPCL-PEVAs. The reversible actuation capability was quantified by cyclic, thermomechanical tensile tests. The experiment consisted of an initial programming cycle and three subsequent reversible actuation cycles.

Results and Discussion

Two well-separated PCL and PE melting and crystallization temperatures, were observed in DSC experiments for cPCL−PEVA5 containing 5 wt% of vinyl acetate (VA). SEM and AFM results revealed a cocontinuous morphology for the prepared crosslinked blends. A uniform overall reversible actuation of $\Delta\varepsilon = 25 \pm 1\%$ was observed having stable nonresponding temperature intervals in both heating and cooling cycles. By varying the heating/cooling rates between 0.5 to 10 K min^{-1} in the thermos-reversible cycles the actuation performance could be tailored. The applicability of this technology was shown in a 140 cycle experiment.

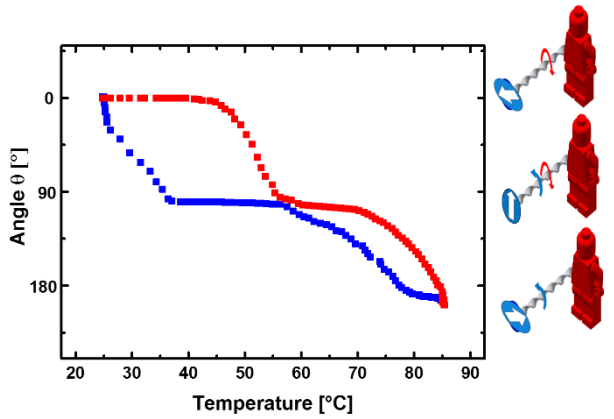

Figure 1. Reversible change in rotation angle of a traffic sign arrow (from 0 to 180 °) mounted on a twisted polymer actuator arm of a manikin as a function of temperature.

Conclusions

In this study, we have demonstrated interruptive actuators based on crosslinked cocontinuous blends of PCL and PEVA. It is anticipated that noncontinuous soft actuators are suitable for autonomously working devices by applying a constant trigger, e.g. scrolling advertisement boards.

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Acknowledgments

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Design of 2D nanosheets reinforced gelatin based electrospun fibers for bone tissue engineering application.

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Introduction

Gelatin is a biopolymer obtained from the partial hydrolysis of collagen. It is biocompatible, biodegradable and very abundant at cheap cost. However, poor mechanical properties of gelatin are the major disadvantage to use it for load bearing application in tissue engineering (1). 2D nanosheets such as graphene oxide (GO) and boron nitride (BN) display high mechanical properties, which were employed to reinforce gelatin nanofibers in this report. We analyzed the influence of 2D nanosheets on electrospun fibers various properties such as mechanical properties, biocompatibility, bone mineralizing biodegradability ability and cell proliferation of electrospun fibers was analyzed.

Experimental Methods

GO was synthesized using the protocol reported elsewhere (2). Various concentrations of GO and BN was dispersed separately in 20% gelatin solution and exfoliated using sonication. Uniform dispersion was used for electrospinning at 25 kV. Fibers were deposited on rotating (400 rpm) aluminum foil. Gelatin/GO and Gelatin/BN electrospun fibers were crosslinked using glutaraldehyde solution for 12 hours and neutralized with 10% glycine solution. Crosslinked fibers were used for further characterizations.

Results and Discussion: Crosslinked electrospun fibers reinforced with GO and BN were stable in aqueous solution and fibrous morphology is maintained which is essential for tissue engineering. Human osteosarcoma cells were cultured on GO/gelatin and BN/gelatin scaffolds as shown in the Figure 1. It proved that the composites are biocompatible. 2D nanosheets reinforced with gelatin improved mechanical property of 100-150% compared to pristine gelatin.

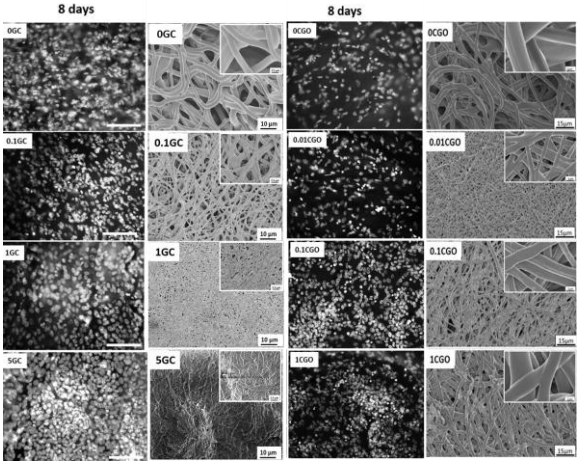

Figure 1. Cell attachment and SEM images of gelatin/GO and gelatin/BN ESM.

Conclusions

Reinforcement of 2D nanosheets improved the nanofiber mechanical property of 100-150%. Electrospun fibers are Biodegradable, stable in aqueous solution, biocompatible and suitable for bone tissue engineering.

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Modulating enzymatic degradation of poly(ε-caprolactone) monolayer at the air-water interface by two dimensional crosslinking

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Introduction

Controlled enzymatic degradation of poly(ε-caprolactone) (PCL) films presents its strong potential for bioactive drugs release system.[1] However, such materials need mechanical stability and degradation kinetics suitable for a particular anatomical site. Such aspects have been attained by crosslinking of bulk PCL.[2] Bulk material surfaces where enzymes interacts, can be fabricated and systematically investigated using the Langmuir technique. For this study, two dimensional (2D) PCL diol monolayers are chemically crosslinked by a dialdehyde and enzymatic biodegradation analysis in real time is explored.

Experimental Methods

(i) 2D network preparation: Oligo (ε-caprolactone) diol (Solvay Caprolactones, U.K.; 2800 g.mol^{-1}) monolayer was cross-linked by injecting glyoxal (Aldrich; molar ratio glyoxal/ caprolactone = 20000/1) into the subphase. The reaction times are reaction times, 5 h and 18 h respectively.

(ii) Degradation by lipase: Lipase from *Pseudomonas cepacia* (0.007 mg.mL^{-1}) is injected into the subphase. Corrected surface area, ΔA_{corr} as function of time was recorded. $\Delta A_{corr}(t) = (A(0) \cdot A(t)^{-1}) - 1$, where A(0) is initial surface area occupied by 2D network at t = 0 and A(t) is required surface area after a certain degradation time interval, t.

Both crosslinking and degradation experiment were performed at surface pressure of highest compressibility modulus, 7 mN m^{-1}; 22±0.5 °C.

Results and Discussion

Lipase catalytic triad cleaves OCL chains into water soluble fragments, causing monolayer area reduction. The slowest degradation rate is observed after 18 h reaction time. Relatively, partially cross-linked OCL monolayer (5 h) degrades in a shorter period. Besides higher mechanical stability, lateral crosslinking modifies hydrophilic -OH end groups and increases polar carbonyl intermolecular linking. In such a dense 2D network, interaction of enzyme with OCL segments is possibly hindered.

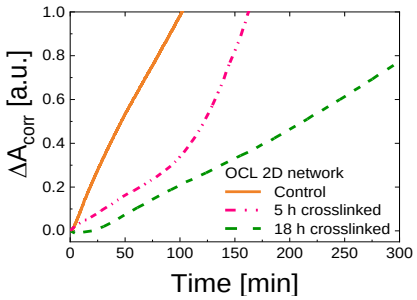

Figure 1. Degradation curve of OCL 2D networks by *P cepacia* lipase.

Conclusions

2D OCL networks prepared as function of crosslinking reaction time displayed slower degradation rates by lipase. Such a model system provides insights into the influence of protein-material interactions by 2D crosslinking.

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PISA prepared Polypeptide Decorated Nanoparticles with Antibacterial Activity

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Introduction

Polymerization-Induced Self-Assembly (PISA), which consists of synthesizing a solvophobic polymer block from a solvophilic polymer block usually using controlled radical polymerization techniques in dispersed media, has revolutionized the preparation of self-assembled nano-objects from block copolymers. Up to date variety of nanoparticles have been prepared through PISA methodology from acrylic block copolymers.

Results and Discussion

Here we report the very first example of polypeptide decorated nanoparticles prepared using RAFT mediated aqueous dispersion polymerization. This work explores the original combination of PISA with functional polypeptides. A short polypeptide consist of 3 Lysines, modified with chain transfer agent is used to conduct dispersion polymerization of hydroxypropyl methacrylate (HPMA) in water at 60 °C. The positively charged polylysine soluble in water acts as the steric stabilizer for the growing insoluble PHPMA chains, resulting in the in situ formation of polypeptide-polymer nano-objects in the form of spheres, worms and vesicles depending on the degree of polymerization of the polymeric block (DP of PHPMA) as judged by transmission electron microscopy and dynamic light scattering studies. The resulting nanoparticles exhibit antibacterial properties due to the presence of the positevly charged polylysine chains on their surface. Thin film membranes were prepared using spin-coating method from a colloidal solution containing peptide decorated spherical nanoparticles. This porous thin film was then used for filtration of contaminated water (water containing Escherichia coli (EC) and Staphylococcus epidermidis (Staph)).

Conclusions

It was observed that both the polypeptide decorated nanoparticle in solution as well as the thin films prepared from them featured antibacterial activity.

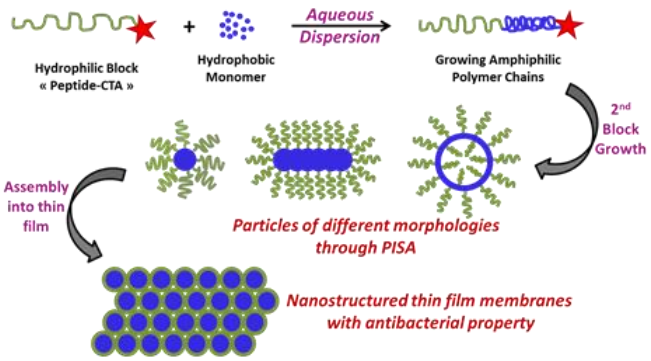

Figure 1. Nano structured membranes from functional block copolymer particles with bactericidal properties.

Seamless biphasic silk construct for the repair of osteochondral defect

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Introduction

The osteochondral (OC) interfaces are one of the most vulnerable areas in the human body to traumatic injuries and diseases such as osteoarthritis. The interface consists of bone and cartilage tissues, which have different molecular compositions, cellular organizations, structural and mechanical properties. For successful osteochondral reconstruction, single intact scaffold should be used to support the regeneration of both cartilage and subchondral bone [1-3]. Therefore, this study aims at developing a seamless silk based biphasic scaffold OC defect repair.

Experimental Methods

Silk fibroin solution and degummed fibers from *Bombyx mori* (mulberry) and *Antherea assamensis* (Indian endemic non-mulberry) silk were used to prepare biphasic scaffolds. The scaffolds were characterized for their physicochemical properties and assesses *in vitro* and *in vivo* using rabbit model.

Results and Discussion

Physicochemical characterization, displayed interconnected pores with differential swelling and tunable degradation. The compressive modulus values, extended to 40 kPa and 25%, for tensile strain at elongation. The scaffold supported growth and proliferation of chondrocytes and osteoblasts, for respective cartilage and bone regeneration. Up-regulation of ALP activity, ECM secretion and gene expression were significant; with acceptable *in vitro* immune response. The implantation in rabbit osteochondral defects for 8 weeks, showed enhanced regeneration of cartilage and subchondral bone tissues. The regenerated bone mineral density ranged from 600-700 mg HA/cm^3.

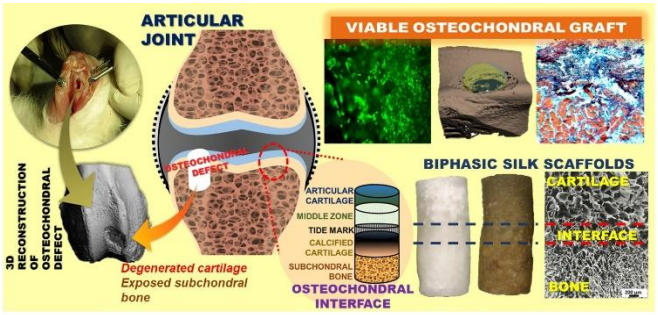

Figure 1. The fabrication, *in vitro* and *in vivo* assessment of biphasic scaffold.

Conclusions

The *in vitro* and *in vivo* assessment establishes competence of all silk, seamless, hierarchical, biphasic scaffolds for repair of osteochondral defects.

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Reversibly Core-Crosslinked PEG-P(HPMA) Micelles: Platinum Coordination Chemistry for Competitive-Ligand-Regulated Drug Delivery

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Introduction

Polymeric micelles have shown great potential for enhancing the solubility and the therapeutic performance of hydrophobic drugs. However, classical non-crosslinked micelles often face the problem of premature disintegration in systemic circulation resulting from dilution in the bloodstream and interactions with blood components.[1] We aim for increased micellar stability via coordinative core-crosslinking by introducing the metal-organic linker [ethylenediamineplatinum(II)]$^{2+}$, herein called *Lx*, in the core of bioresorbable poly(ethylene glycol)-(N-(2-(4-(methylthio)benzoyl)oxypropyl) methacrylamide) (PEG-P(HPMA-MTB)) micelles.

Experimental Methods

PEG-P(HPMA-MTB) diblock copolymer was synthesized via free radical polymerization of HPMA-MTB monomer initiated by $(PEG)_2$-ABCPA macro-initiator for 18 h at 70 °C in methanol. For preparation of core-crosslinked micelles, PEG-P(HPMA-MTB) was coordinated with *Lx* in DMF at 37 °C for 48 h, followed by evaporation of DMF. The polymer-linker conjugates were subsequently dissolved in a mixture of acetone and water to allow formation of core-crosslinked micelles via coordination chemistry, followed by evaporation of the acetone. Non-crosslinked micelles were prepared by adding a THF solution of polymer into water followed by evaporation of THF.

Results and Discussion

The critical micelle concentration (CMC) of *Lx* crosslinked micelles (termed *Lx* PEG-P(HPMA-MTB) micelles from here on) was found to be 0.009 w/v %, which is lower than the CMC of non-*Lx* PEG-P(HPMA-MTB) micelles (0.03 w/v %). The tendency of the polymers to form micelles at lower concentrations in the presence of *Lx* linker is a strong indication for *Lx* mediated coordinative core-crosslinking. Moreover, DLS measurements showed a shift of the micellar size to smaller dimensions upon the introduction of *Lx* from 160 nm to 60 nm, reflecting a more condensed core in the micelles due to the coordinative crosslinking. The *Lx* PEG-P(HPMA-MTB) micelles showed a better retention of the versatile, hydrophobic drug curcumin during *in vitro* release experiments in comparison with non-*Lx* PEG-P(HPMA-MTB) micelles. When the reducing agent dithioerythritol (DTE) was added to mimic the reductive intracellular environment, the release of curcumin from *Lx* PEG-P(HPMA-MTB) micelles was significantly accelerated due to competitive coordination of the platinum atom in *Lx* with DTE, resulting in micellar destabilization.

Conclusions

We report on a straightforward and original method for the preparation of stable micelles which are reversibly core-crosslinked via platinum coordination chemistry.

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Protein-polymer interaction in Langmuir monolayers: the study of PhaF protein binding to polyhydroxyalkanoates

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Introduction

Bacterial polyhydroxyalkanoates (PHA) are *in vivo* polymerized polyesters with the potential to outperform other polymeric materials in medical applications due to their natural origin, and controlled biodegradability [1]. To enhance cell-material interactions, the functionalization of polymers surfaces with biomolecules has been accomplished [2]. PHA-producing bacteria also produce phasin PhaF, an amphipathic protein, which generates an interface between the cytoplasm and the hydrophobic core of PHA. *In vitro,* PhaF would provide the PHA surface with a more hydrophilic character thereby decreasing non-specific interactions with biological environments. The aim of this work is to study the organization and stability of PHA films at the air-water interface and to get an insight into the interactions of PHA with PhaF at the molecular level.

Experimental Methods

Poly(3-hydroxyoctanoate-co-3-hydroxyhexa-noate) (PHA) was synthesized by bacterial fermentation of *P. putida* KT2440 (Mw= 166 400 g·mol^{-1}). PhaF was purified by affinity chromatography (GE Healthcare Life Sciences) from recombinant *E. coli*. Pressure-area isotherms were measured using a Langmuir trough with a custom-made level compensation system. (KSV LTD, Finland). Structural changes of the layers were recorded by infrared reflection absorption spectroscopy (IRRAS, KSV LTD).

Results and Discussion

At the air–water interface, PHA forms thin films of 1-2 nm thickness that collapse during film compression at a surface pressure of π = 15 mN×m^{-1}. The formation of circular aggregates traced by Brewster angle microscopy is reversible when the monolayer is expanded (Fig. 1). PhaF injection (0.5 µg×.ml^{-1}) underneath PHA monolayer (π = 7.5 mN×m^{-1}) increases ≈10% the film area, indicating the formation of a mixed layer. Protein adsorption is confirmed by the presence of amide bonds in IRRAS spectrum and the conformational analysis of PhaF.

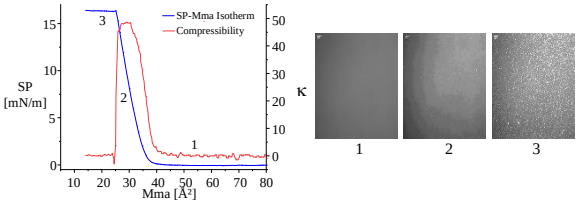

Figure 1. Pressure-area isotherm and BAM images of PHA at the air-water interface.

Conclusions

It is possible to mimic the PHA natural environment by applying the Langmuir-Blodgett technique to demonstrate the PhaF adsorption at the polyester layer. The results confirm the potential of the PHA surface for modification *via* hydrophobic adsorption of PhaF, to produce a more hydrophilic material for medical applications.

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3D-biopring of peptide based hybrid organic-inorganic hydrogels: Encapsulation of mesenchymal stem cells for cartilage repair

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Introduction

With settlement and aging of population, more and more people suffer from cartilage or bone damage. 3D-printing techniques may be used to prepare scaffolds and prosthesis to treat articulation defects [1]. One of the main challenge is to develop bioinks, suitable for cells encapsulation and whose composition can be adapted to each type of tissue before being degraded and replaced by native extracellular matrix. In this context, we developed biomimetic inorganic/bioorganic hydrogels composed of different hybrid silylated blocks (polymers, peptides, dyes…) that can be printed by extrusion using sol-gel inorganic polymerization.[2, 3]

Experimental Methods

The hybrid organic-inorganic blocks used to prepare the hydrogel is bisilylated –polyethylene glycol in the first part to optimize sol gel process, then a bisilylated – synthetic collagen peptide. Peptides were prepared by solid phase peptide synthesis. Hydrogels were prepared by a sol-gel process in culture medium, with biocompatible amount of catalyst. Viscosity of hydrogel is monitored using an A&D vibro viscometer.
Mouse Mesenchymal stem cells are encapsulated into the hydrogel, before 3D printing by an extrusion process with a BioBot printer.

Results and Discussion

After many viscometric studies, we found that temperature, catalyst, pH but also medium, nature of the silylated block and presence of cells have a strong impact on the sol gel process.
We manage to 3D-print this hydrogel, according to a printing window of viscosity.
Biological assays show a good viability of the cells encapsulated in the hydrogel and a good proliferation, compatible with a biological application for cartilage repair.

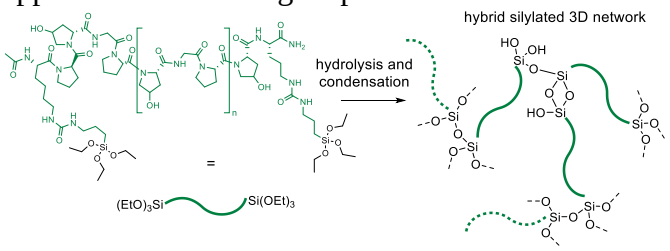

Figure 1. Sol gel process giving hydrogel

Conclusions

We manage to prepare a biocompatible hydrogel printable by extrusion, which could be used for cartilage repair but also as a platform for any 3D printing with biological application.

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Laurine Valot's PhD was founded through an ANR grant attributed to G. Subra, A. Mehdi and D. Noël. Peptide synthesis and purification were performed using SynBio3 platform facilities.

Collagen type-IV Langmuir-Schaefer films as substrates to direct mesenchymal stem cell adherence

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Introduction

A significant challenge in the field of biomaterials is to control the layer of proteins that are formed at the material interface both *in vitro* and *in vivo*, in order to achieve targeted cell responses. The Langmuir-Schaefer (LS) method offers the possibility to control the protein layer characteristics at the material interface such as thickness, orientation and distribution of cell adhesion sequences.[1, 2] Collagen-type IV (Col-IV), the major scaffolding component of basement membranes, has the propensity to form networks by self-assembly and supports cell adhesion of many cell types including stem cells.

Experimental Methods

LS films were prepared in a Langmuir trough (KSV-NIMA) and Col-IV from human placenta (Sigma-Aldrich) was spread on the subphase pH 7.5 + 100 mM NaCl, and after 30 min stabilization time, and barriers were compressed to the three different target pressures and LS transfer was performed using PET cover slips (Nalge, Nunc). Adipose derived human MSCs were seeded on PET surfaces equipped with Col-IV for 24h and focal adhesion (vinculin) and cytoskeleton formation (F-actin) was visualized using immunostainings and confocal microscopy. (510, META, Carl Zeiss).

Results and Discussion

Surface pressure area isotherms show that the concentration of Col-IV molecules can be controlled by surface compression and one can identify the monolayer collapses around a surface pressure of 35 $mN \cdot m^{-1}$. (Fig. 1a) Furthermore, as the density of Col-IV was increased more cells were adherent on PET substrate. There was also strong cell adhesion on PET modified with solution deposition (SO) in comparison to the bare substrate. However, the Col-IV layer on SO is rather uncontrolled with formation of aggregates and multilayers. (AFM data not shown).

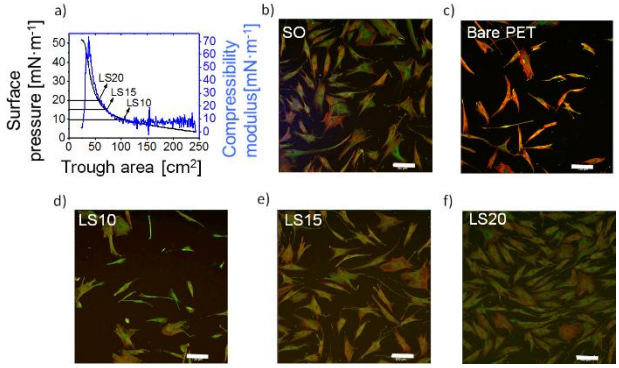

Figure 1. a) Surface pressure and compressibility modulus vs trough area. b-f) Stem cell adhesion on PET equipped with Col-IV by LS and SO. (Scale bar: 100 µm)

Conclusions

Langmuir-Schaefer technology can be used to control the molecular density of Col-IV networks on PET and thereby, direct the stem cell adherence.

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Design of Biodegradable Anti-adhesive Membranes in Orthopedic Surgery

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Introduction

Post-surgical tissue adhesions are late complications in several surgical specialities including upper limb tendon and nerve orthopedic surgery. A promising approach to prevent adhesions is the implantation of a polymeric physical barrier at the injured site[1,2].This work aims at synthesizing a multi-degradable high molecular weight polyethylene oxide by synthesizing a polyether urethane (PEU).[3]

Experimental Methods

All reagents were purchased from Sigma Aldrich and were used as received.Polyethylene glycol (PEG) was dried by azeotropic evaporation with toluene. Polyether urethane (PEU) was synthesized by a polycondensation process with1,6-hexamethylene diisocyanate (HMDI).Poly-(lacticacid)-b-polyether urethane-b-poly (lactic acid) (PLA-PEU-PLA) triblock copolymer was synthesized by a ring opening polymerization pathway.

Results and Discussion

Polycondensation of PEG with HMDI allowed to synthesize PEU as shown in Figure1.

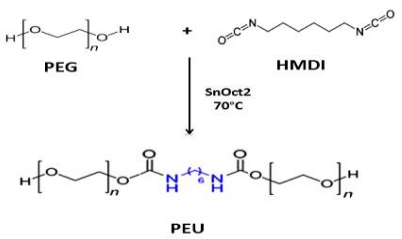

Figure1.Synthesis of polyether urethane

Starting from a PEG 2000 Da, Gel Permeation Chromatography analysis of the related PEU showed a molecular weight of 25000 Da and a polymerization degree around 12. Infra-red analysis confirmed the formation of the urethane groups. PLA-PEU-PLA triblock copolymer was synthesized by a ring opening polymerization of D,L-lactide**.**1H-NMR and GPC analyses allowed to determine the copolymer composition: PLA blocks of 92000 Da for a PEU block of 25000 Da.

Conclusions

Multi-degradable PEO, as polyether urethane (PEU), has been successfully synthesized by a one-step polycondensationroute. Based on this PEU, PLA-PEU-PLA copolymers were prepared. PEU degradation kinetics are currently being studied. PLA-PEU-PLA membranes will be then prepared to study their mechanical properties as well as their cytocompatibility and their degradation properties.

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Tuning polymer elasticity to regulate human mesenchymal stem cells: a potential strategy for tissue-engineering of blood vessels

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Introduction

The poly(n-butyl acrylate) network (cPnBA) demonstrates a high potential as synthetic vascular grafts owing to its low toxicity and tailorable compliance matching various native blood vessels [1, 2]. Mesenchymal stem cells (MSCs) are an attractive cell type for accelerating endothelialization because of their superior anti-thrombosis and immune modulatory function. Further, they can be differentiated into smooth muscle cells or endothelial-like cells and secret pro-angiogenic factors such as vascular endothelial growth factor (VEGF). Here, the influence of the elastic properties of cPnBA on the cellular response of MSCs is studied.

Experimental Methods

The cellular response of human adipose-derived stem cells (hADSCs) on cPnBA films with elastic modulus matching the native artery (with either small or large diameter) was assessed via ELISA and time-lapse microscope.

Results and Discussion

cPnBA showed high compatibility for hADSCs. Although F-actin assembly of hADSCs was decreased on cPnBA films compared to traditional tissue culture plate. The difference of cPnBA elasticity did not show effects on cell attachment, morphology, cytoskeleton assembly, apoptosis and senescence. Cells on cPnBA250 (E = 250 kPa), with lower proliferation rate, had significantly higher VEGF secretion (Fig. 1) and faster migration velocity than cells on cPnBA1100 (E = 1100 kPa). The potential mechanism of MSCs' elasticity sensing might due to the activation of integrin-mediated ROCK/ERK signaling pathway and further enhanced VEGF secretion [3, 4].

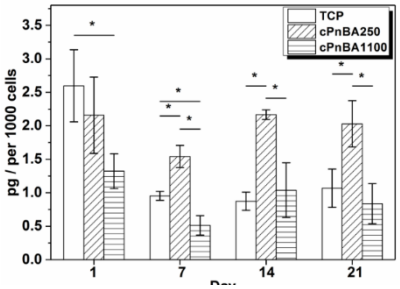

Figure 1. VEGF secretion of hADSCs on tissue culture plate (TCP) and cPnBA films.

Conclusions

These results demonstrated the high cell compatibility of cPnBA as synthetic vascular grafts. Tuning their elastic profile can affect MSCs paracrine activity, which might further improve the endothelialization and long-term engraftment of vascular graft.

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