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Young Scientist Forum

S05-01

Towards Bioprinting of Biphasic Scaffolds for Bone Defects: Multichannel Printing of a Calcium Phosphate Cement and a Cell-laden Hydrogel

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Introduction

Extrusion based additive manufacturing allows printing of cells within a cell convenient hydrogel, which commonly is referred to as bioprinting. Although the stiffness of hydrogels can be tailored, hydrogels might never reach the stiffness required for bone tissue engineered constructs. We have demonstrated the extrusion of plottable calcium phosphate cements (CPC) at mild conditions (A Lode et al., J Tissue Eng Regen Med, 2014); After transformation of the precursors to hydroxyapatite (HAP), the cement showed both high cell compatibility and mechanical stiffness suitable for bone scaffolds. Herein, the CPC was co-extruded with a cell-laden hydrogel blend consisting of 3% alginate and 9% methylcellulose (Alg-MC) (Schütz et al., J Tissue Eng Regen Med, 2017) to achieve bioprinted scaffolds with macropores and increased stiffness.

Materials & Methods

CPC (Velox, INNOTERE, Radebeul, Germany) and Alg-MC were prepared as described in the literature and processed with a multichannel printing system (Bioscaffolder 3.1, GeSiM, Großerkmannsdorf, Germany). Human mesenchymal stem cells (hMSC) were mixed into the Alg-MC material. Printed constructs were analysed microscopically (stereo light microscopy, SEM, cLSM, fluorescence microscopy) and by uniaxial compressive tests.

Results & Discussion

Both materials were successfully co-extruded and combined to one scaffold. The optimal conditions for CPC setting and alginate crosslinking within the biphasic scaffold were investigated. If immersed into liquids, CPC hardens but microcracks arise simultaneously which does not happen in case of setting in humidity (Akkineni et al., Acta Biomater, 2015). Herein we observed, that initial setting for 20 min is sufficient to minimize the appearance of microcracks. Furthermore, cell viability of hMSC inside non-crosslinked Alg-MC scaffold incubated in humidity up to 30 min was not changed. As a result, biphasic scaffolds after printing were incubated in humidity for 20 min, followed by crosslinking of the hydrogel and incubation in cell culture medium. At day 1 after post-processing of the biphasic scaffolds, the cell viability was significantly reduced at the interface region between the two materials; after 21 d cells migrated from the hydrogel to the CPC strands. In conclusion, a method was developed for the fabrication of biphasic scaffolds comprising CPC and a cell-laden hydrogel, as a basis for fabrication of constructs for bone and osteochondral regeneration.

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S05-02

Hydrogels as Carriers for Photosensitizers Used in Photodynamic Therapy

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Hydrogels are water-swollen polymer networks that can be loaded with bioactive substances and drugs very easily. They can absorb a large amount of water and possess a high degree of flexibility.[1, 2]

In this study we use poly(ethyleneglycol) diacrylate hydrogels. These are promising materials for biomedical applications such as drug delivery systems.[3-5] The produced hydrogels will be used as wound patches for infected or chronic infected wounds. Therefore, so called photosensitizers shall be incorporated in the hydrogels. These photosensitizers can act as antimicrobial substances due to singlet oxygen generation after irradiation with light. [6, 7]

To produce those antimicrobial wound patches the hydrogels were loaded with various well-known photosensitizers, e.g. methylene blue, eosin y, and porphyrins. Their uptake and release kinetics were observed. In Figure 1 the high transmittance over a broad wavelength range of the gels is shown. This property is very important to be sure the photosensitizers can be irradiated after incorporation in the hydrogels.

Additionally, the hydrogels were investigated by different analyzing methods, e.g. scanning electron microscopy (SEM), infrared spectroscopy (IR), UV/VIS spectroscopy, and dynamic mechanical analysis. Furthermore, properties like swelling behavior and mesh size were determined.

The method shown here is a simple and effective preparation and loading strategy for wound gels. These are great carriers for photosensitizers. Especially with respect to the growing number of antibiotic resistances, those gels are highly interesting for future medical applications.

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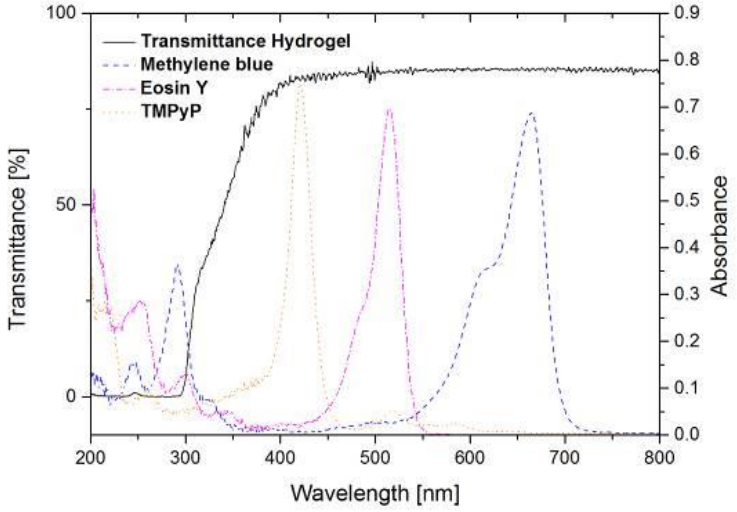
Figure 1



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S05-03

3D-electrode scaffolds enabling cell infiltration and seamless tissue integration

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Introduction

The formation of capsules around electrode implants due to the foreign body response is still the central limitations of current pacemakers. Inflexible materials and absence of cell infiltration are mentioned as main reasons for this incompatibility. The generation of tissue-like fiber electrodes and an embedding in host cells is a promising way for an effective tissue integration and energy transfer.

Methods

Porous 3D carbon nano fiber scaffolds were generated by electrospinning of polyacrylnitrile and strewing of NaCl particles during the spinning process. Subsequently, the scaffold was stabilized and carbonized resulting an electrical conductive and porous electrode. The properties of the porous scaffolds were defined by SEM, electrical and mechanical characterization. Cell-infiltration studies were performed with fibroblasts (hdF) until 12 weeks. The generation of hybrid electrodes containing functional cardiac tissue was performed in a complex long-term co-culture system.

Results

SEM analysis of the porous scaffolds revealed in an extended fiber network with increased mesh-openings up to 75 µm² compared to standard electro-spun scaffolds. Cross sections demonstrated remaining pores with a height of about 40 µm and several 100 µm² in length. Bending tests showed a high flexibility, with no failure in all tested diameters for the scaffolds. HdF cell culture confirmed the migration of the cells through the scaffold in a time frame of 6 to 8 weeks. Cardiomyocyte (CM) cell culture revealed the scaffolds compatibility by forming contracting cell aggregates. Extending the culture with a combination of CM, hdF and MSC resulted in a hybrid electrode with functional cardiac tissue. Electrical stimulation and histological stainings visualized the functionality of the hybrid electrode. Subsequently, the hybrid electrode was placed on an *in vitro* cardiac tissue model. After 4 weeks, the generated construct composed of cardiac model and hybrid electrode was analysed by stimulation, force measurements and histology.

Conclusion

3D porous and flexible nano fiber electrodes enabling cell migration were developed. The compatibility and possibility for infiltration of specific cells provides a new approach of hybrid electrode systems. The ability of a seamless integration in a specific tissue, the formation of a functional unit, strengthens this combined approach of material and tissue engineering for the development of tissue-electronic interface.

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S05-04

Dual release from core-shell nanoparticles of nanoporous silica and nanoporous organosilica

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Drug delivery from nanoporous particles is an important field of research. Nanoporous silica nanoparticles (NPSNPs), often equipped with surface modifications to regulate drug release, have already been investigated for a wide range of applications. [1] The first investigations using nanoparticles of PMOs have also appeared. [2-4] In general, such nanoparticle systems are able to release only a single active agent, although in many cases it would be advantageous to deliver a combination of different drugs, possibly sequentially in time. For example, when implant-associated drug release is concerned, delivery of an anti-inflammatory/antibacterial drug followed by an active agent to promote healing would be beneficial.

This work focusses on the synthesis and characterisation of smart hybrid core-shell nanoparticles, which can be further developed to perform a sequential release of different drugs over several months. As core material, NPSNPs are used which have already been positively tested as drug delivery systems and the biocompatibility of which has been proven in cell culture tests. The shell of the particles consists of a periodic mesoporous organosilica (PMO). The diameter of the NPSNPs is 30-40 nm; the complete core-shell nanoparticles have a size of 80 nm (Figure 1). Remarkably, the two parts of the particles feature different chemical character, the nanoporous silica being hydrophilic, whereas the shell is more hydrophobic due to organic bridging units. The core-shell nanoparticles should therefore lend themselves to dual drug release of hydrophilic (from nanoporous silica core) and hydrophobic (from PMO shell) drugs. Due to the hydrophobic shell, the release of the molecules adsorbed in the core should be slower than the release of the drug from the shell. In an ideal case, these would be separated in time. In our initial studies, we tested dyes as guest molecules. Afterwards, different drugs were used and biomedical applications will be tested.

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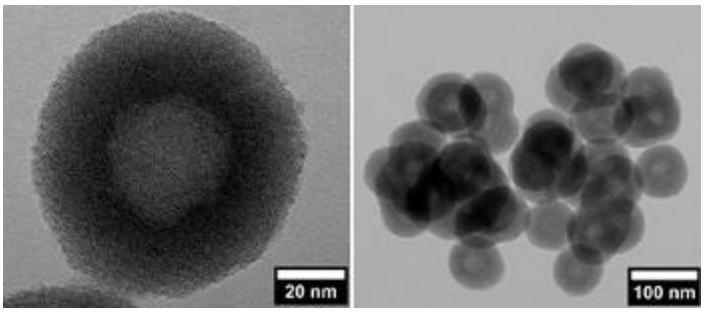
Figure.1: TEM images of nanoparticles with porous silica core and porous PMO-shell.

Figure 1



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S05-05

Amphiphilic Poly(Oxazoline)s forming biocompatible, thermoresponsive, and injectable gels for multiple biomedical application

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The placement of cells within biomaterials into spatially defined structures, better known as 3D bioprinting, finds increasing interest among engineers, scientist and clinicians.[1] A market research conducted by IDTechEx forecasts that the market for 3D bioprinting will reach $1.8 billion by 2027. With this predicted development and the ever increasing visions on potential and actual applications, the need for suitable and tunable hydrogels can be seen as a potential bottleneck. The so-called Bioinks have to fulfill various requirements like consistent quality, sufficient quantity and customizable biological and physical properties.[2] Furthermore, the forces that occur during the printing process need to be mimicked and their effects on the material properties as well as on the cells have to be investigated. Therefore, natural polymers as well as synthetic polymers need to be taken into account as both have advantages and disadvantages.

One polymer class that until recently was not taken into account as potential bioink are poly(2-oxazoline)s, a prominent member of pseudo-polypeptides.[3] In the last decades, they have been intensely investigated especially as thermoresponsive materials[4] and for biomedical applications.[5] More recently, polypeptoid based hydrogels were reported.[6] Here we report the synthesis of novel thermogelling pseudo-polypeptides, their cytocompatibility and structure property relationships with respect to their rheological properties. Additionally, we present our latest results regarding cell survival during the printing process. Furthermore, small angle neutron scattering revealed an interesting nanoscopic structure, which will be discussed and compared with other thermogelling polymers based hydrogels.

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S05-07

Autofluorescent ultra-small gold nanoparticles as optical labels for the cell uptake of polymeric nanoparticles

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Introduction

Nanoparticles are an interesting tool for drug delivery into cells. Ultra-small gold nanoparticles (<2 nm) have a high specific surface area and are autofluorescent, making them useful for a label-free detection inside cells. To investigate their potential, the gold nanoparticles were encapsulated into larger nanocapsules of the biodegradable polymer poly(D,L-lactide-*co*-glycolide) (PLGA).

Methods

Ultra-small gold nanoparticles were prepared in a one-pot synthesis by reducing tetrachloroauric acid with sodium borohydride in water, followed by stabilization with 11-mercaptoundecanoic acid.[1]

The gold-loaded PLGA-nanocapsules were prepared by a water-in-oil-in-water (W1/O/W2) emulsion solvent evaporation technique,[2] using the gold nanoparticles in the W1-phase, PLGA in dichloromethane as O-phase and polyvinyl alcohol (PVA) in water as W2-phase. To further enhance the cellular uptake of these particles, they were coated with polyethyleneimine (PEI), reversing their charge from negative to positive. Cell uptake studies were carried out with HeLa cells, during incubation for 3 and 24 h.

Results

By differential centrifugal sedimentation (DCS), an average diameter of 1.8 nm was determined for the gold nanoparticles. Fluorescence spectroscopy shows an emission maximum at a wavelength of 620 nm, making them suitable for intracellular detection by confocal laser scanning microscopy. The average PLGA capsule diameter was 125 nm (SEM). STEM-imaging showed that the PLGA formed hollow capsules with incorporated gold nanoparticles. After surface charge reversal, the zeta potential of the PLGA capsules was +30 mV. Cell uptake studies showed the capsules on the cell membrane after 3 h and inside the cell after 24 h.

Conclusion

Gold nanoparticles can be encapsulated into PLGA. Due to their autofluorescent nature, additional optical labelling, e.g. by an organic fluorescent dye, is not necessary for biological uptake studies. After an incubation time of 24 h the PLGA capsules were taken up by the cell and detected by the autofluorescent gold nanoparticles.

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S05-08

Antibacterial efficiency of silver sacrificial anode thin films within a bacteria-containing plasma clot as an in vitro model for infected tissue

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Infections related to medical devices and implants cause constant and serious clinical problems. Bacteria can adhere to and colonize the surfaces of various biomaterials. Uncontrolled bacterial growth may result in biofilm formation which protects the microorganisms against host defense mechanisms and makes antibiotic treatments difficult if not ineffective. Many strategies have been followed to inhibit initial bacterial colonization on surfaces, either by specific topography or by special coatings such as silver in various applications. Recently, we presented a sacrificial anode principle as a new approach to achieve enhanced release of silver ions from Ag thin film coatings on gold or platinum-element layers (platinum, palladium, and iridium) and demonstrated enhanced antimicrobial activity of the sacrificial anode samples compared to similar silver depositions on titanium. Analyses of antimicrobial Ag coatings or Ag nanoparticles are often performed in fluid-based systems such as conventional cell culture. Generally, protein concentration and fluid diffusion is clearly different e.g. in muscle tissue or connective tissue compared to cell culture medium. Thus, the objective of this study was to establish a tissue-like matrix which could be loaded with growing bacteria and to analyze the antibacterial capacity of different Ag coatings inside this matrix. Therfore, Ag dots arrays (64 and 400 dots per mm2) are fabricated on a continuous platinum, palladium, or iridium thin film (sacrificial anode system for silver) and for comparison also on titanium film (non sacrificial anode system for silver) by sputter deposition and photolithographic patterning. In addition, dense silver films of same size are produced. The samples are embedded within a tissue-like plasma clot matrix containing growing Staphylococcus aureus (S.aureus), cultivated for 24 h and bacterial growth was analyzed by fluorescence microscopy. Among platinum group sacrificial elements and the dense silver sample only the high Ag ion releasing Ir-Ag system was able to inhibit the bacterial growth within the plasma clot matrix. Therefore, it is necessary to develop Ag coatings which are still effective even under tissue-like conditions such as the Ag-Ir- sacrificial anode system which might be at least temporally able to keep an implant sterile.

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S05-09

Easy-to-prepare Poly(2-n-propyl-oxazoline) coatings of standard cell culture dishes from aqueous solutions for Cell Sheet Engineering

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Cell sheet technology is a well-known method based on culture dishes covalently grafted with a monomolecular thin layer of a thermoresponsive polymer exhibiting a lower critical solution temperature (LCST) in water, commonly poly(N-isopropylacrylamide) (PNIPAAm). The critical temperature for PNIPAAm is 32° C, meaning that above 32° C, PNIPAAm chains are dehydrated, leading to hydrophobicity of the grafted dish surfaces so that cells can adhere and proliferate at 37°C. Below 32° C, the polymer reversibly changes to hydrophilic properties, meaning that cells detach from the surface as complete sheets, including the matrix they produced.

Besides PNIPAAm, other polymers also show LCST behavior in water, e.g. polymers based on poly(2-oxazolines) (POx). POx are known as smart polymers because of their tunable solution properties and biomedical applications, therefore playing an emerging role in the field of life sciences.

In this study, we examined whether a covalent coupling of polymer layers on surfaces is necessary for cell sheet preparation, or whether an easy to apply procedure can be established that can be transferred to standard cell culture well-plates and prepared in each standard biological laboratory. To examine this, different standard cell culture dishes were repeatedly covered with 0,1% aqueous solutions of poly(2-n-propyl-2-oxazoline) (PnPrOx) and dried in the oven for drying to create a fully covered thermoresponsive surface. Contact angle measurement was performed to analyze the thermoresponsive characteristic of the coating. Above the LCST of PnPrOx, a hydrophobic contact angle of 70° was determined, by decreasing the temperature, the contact angle change to a more hydrophilic state with 48°. Furthermore, different cell types were seeded and culture until confluency on PnPrOx-covered surfaces. By decreasing the temperature to 16° C for 1-2 hours depending on the cell type, cell sheets detached and could be harvested and used for cell sheet technology approaches. We tested different cell types for their potential to generate cell sheets via this method and analyzed the sheets via histological stainings, proving the functionality of the cell sheet.

With this method, we established an easy, user-friendly and low-cost variation of creating thermoresponsive surfaces applicable for cell sheet technology and tissue engineering purposes by simply covering surfaces of different types of dishes with PnPrOx solved in water and drying afterwards.

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S05-10

"Subsurface Imaging" of magnetic nanoparticles and quantification of nanomechanical properties of polymers and biological materials by bimodal atomic force microscopy.

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In targeted drug delivery, the active pharmaceutical ingredient is released directly at its place of destination. The efficiency of its effect can thus be raised. This, for example, is used for cancer treatment. However, the exact motion processes of such nanoparticles in cell tissue are not yet fully understood. A tool is required to directly visualize the motion processes and to simultaneously map the local mechanical properties of the target.

We aim to observe the intrusion process of nanoparticles into human cells by bimodal frequency-modulated atomic force microscopy. To this end, a magnetic tip of an atomic force microscope is used to simultaneously detect the local mechanical properties of human HUVEC-cells and the subsurface locations of superparamagnetic ferritin nanoparticles.

We show that the mechanical characterization of biologic and polymeric matter is possible by the use of low-noise small cantilevers and photothermal excitation of the cantilever. To detect magnetic properties in a liquid environment, we increased the cantilever"s oscillation amplitude several times using a backside coating of colloidal graphite. By embedding ferritin nanoparticles into a polymer-film, we studied the depth limits of the subsurface detection. High-resolution measurements of ferritin nanoparticles in liquids of different pH-values provided a first indication of the stability and organization of the protein shell. Moreover, we present images of human HUVEC-cells acquired by dynamic force microscopy.