Poster

P21

Prevention of ureteral catheter encrustation - a comparison of multiple polymer bulk materials

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Introduction

Ureteral catheters are used for temporary as well as long-term stenting of the ureter. Complications of polyurethane catheters, like biofilm formation or encrustation, promote urinary tract infections and cause disorders in wound healing in the ureter. Encrustations are triggered by the precipitation of salt crystals, and are always accompanied and often caused by bacterial infections. We aim at developing a new polymer catheter that should minimize encrustation, prolong the laytime and thus reduce the burdens of the patient.

Materials

We are testing substrates of different polymer types [Thermoplastic Urethanes (Elastollan, Tecophilic), Styrene-Butadiene Copolymers (Styrolflex), Ethylen-Vinylacetat Copolymers (Greenflex)] and determine the chemical characteristics (water contact angle, surface energy and charge) as well as encrustations. Chemically defined synthetic urine [1] with increasing pH is dynamically pumped over the samples with defined stop-go cycles and adjusted flow rates at 37°C to mimic the native body urine flow. The encrustations are analyzed quantitatively and qualitatively using scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy and Fourier-transform infrared spectroscopy.

Results

We developed an in vitro encrustation system in which we were able to provoke encrustation on polymer samples within five days (Fig1). The morphology and quantity of the encrustation is dependent on the surface characteristics of the material (highest Tecophilic>Styrolflex>Greenflex>Elastollan lowest).

Conclusion

We suggest considering encrustation experiments for the development of new ureteral catheters to minimize encrustation, hence decreasing the potential growth surface for bacteria.

[1] Griffith et al. (1976) Invest Urol, 13(5):346-50.

Fig1: SEM image of an encrusted Tecophilic surface after 5d in the *in vitro* encrustation system. Note the mix of amorphous depositions and clear-cut crystals. SEM Merlin VP, Zeiss

Figure 1

A modified shell-less hen's egg test to evaluate the irritation potential and antimicrobial activity of cold atmospheric plasma (CAP)

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Question

There is a high unmet medical need for alternative antimicrobial treatments and test systems due to the growing rate of antibiotic resistances. A promising approach is the use of CAP. For investigation of antimicrobial efficacy and biocompatibility *ex ovo*, a shell-less hen's egg test on the chick area vasculosa (HET-CAV) was established.

Methods

An argon plasma jet (kINPen® 09) and a DBD (dielectric barrier discharge) plasma source based on low temperature cofired ceramic technology (IlmPlas) were compared. Fertilized hen's eggs were incubated for 72 h and transferred into petri dishes. Plasma was directly applied on the vessel system CAV for different exposure times to investigate irritative effects (hemorrhage, vascular lysis, thrombosis, lethality) up to 24 h. *P. aeruginosa* (PA) was further co-cultivated on the CAV, followed by CAP treatment to determine survival rate over 68 h. PA was cultivated on MH2-agar plates and treated with CAP to evaluate antimicrobial activity *in vitro* by formation of zones of inhibition (agar diffusion test).

Results

Treatment of hen's egg with the plasma jet caused hemorrhagic events and vascular lysis followed by lethal events, whereas the DBD plasma demonstrated high compatibility with only sporadic hemorrhagic reactions. Hence, further antimicrobial studies were performed with the DBD plasma. Without treatment, co-cultivation of PA on the CAV caused strong hemorrhagic reactions and embryonic lethality within 24 h. Treatment of PA with the DBD plasma increased the survival rate of the chick embryo depending on exposure times. The *in vitro* agar test confirmed the good antimicrobial efficiency.

Conclusions

The modified hen's egg test allows the determination of antimicrobial activity and biocompatibility of novel CAP sources for biomedical applications in a complex biological system. An efficient antimicrobial activity accompanied with a good biocompatibility was observed for the DBD plasma source.

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Figure 1

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Short-term magnetron sputtered silver-platinum nanopatches as anti-bacterial sacrificial anode systems

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Infections related to medical devices and implants cause constant and serious clinical problems. Uncontrolled bacterial adherence and 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 dot arrays on platinum-element layers and demonstrated enhanced antimicrobial activity of the sacrificial anode samples compared to similar silver dot arrays on titanium.

To further reduce the total amount of coated silver as well as the rare and expensive platinum-group metals a very short sputter time (10s, 20s or 60s) was applied which resulted in nanopatch-like deposits (5-60 nm in size, dependent on sputter time). Three different sample types were fabricated: silver patches, consecutively sputtered platinum and silver patches, and co-sputtered platinum/silver patches. Antimicrobial activity was tested towards *S. aureus* using a drop-based assay which allowed the detection of adherent and planktonic bacteria.

The results demonstrated enhanced antimicrobial activities of the samples which are coated with the sacrificial anode Ag-Pt patches compared to the silver patches. The antimicrobial effects were self-limited due to the dissolution of silver from the nanosized deposits.

Our results demonstrate an active sacrificial anode effect induced by nanopatch structures (Ag patch in contact to Pt patch) and even induced by single co-sputtered (Pt-Ag) nanopatches. Such decorated implants may be effective to avoid bacterial adherence concomitantly to a noble metal sparing strategy.

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Antibiotic-loaded bone allografts for prophylaxis and treatment of bone infections

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Introduction

Every surgical procedure is accompanied by a risk of bacterial infections especially in transplantations of bone allografts for regeneration of lost tissue in dental and orthopedic applications. One particular challenge is the population of bioimplant surfaces with biofilm-forming bacteria, which are less prone to antibiotic treatment [1]. The loading of bone allografts with antibiotic agents demonstrates a promising approach to overcome this issue. In the present study the pharmacokinetic properties of several antibiotics incorporated into allografts were analyzed *in vitro* and *in vivo*.

Design and Methods

Freeze-dried bone allograft (FDBA) blocks (C+TBA, Krems, Austria) were rehydrated in antibiotic solutions of either Clindamycin, Gentamycin, Rifampicin, Vancomycin and a mixture of Vancomycin and Rifampicin for biofilm treatment for 10 minutes in a 1:1 proportion. The *in vitro* concentration of remaining antibiotics was assessed every 24 hours for up to 10 days. For analysis of the *in vivo* release of the antibiotic-loaded blocks were implanted using the tibial implantation model in 30 rabbits. The allografts were explanted at day 1 and 3 and the remaining amounts of the antibiotics were analyzed using microbiological methods.

Results

Antibiotics are easily incorporated into FDBA blocks and especially within the first 3 days high concentrations were released both *in vitro* and *in vivo*.

Conclusion

The use of FDBA as carriers of antibiotic agents bares great potential in clinical application by eliminating systemic antibiotic-related side effects, minimizing risks of antibiotic resistance formation, providing advantages for eukaryotic cells for faster surface population and consequently minimizing the risk of surgical site infections.

[1] Stewart, Philip S., and J. William Costerton. "Antibiotic resistance of bacteria in biofilms." The lancet 358.9276 (2001): 135-138.

P26

Bacterial cell division is involved in the physico-mechanical destruction of gram-negative bacteria on a nano-pillar titanium surface

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Introduction

The development of biomaterials preventing bacterial colonization is an important challenge in biomaterial research. Nano-pillar surfaces on Cicada wings exert bactericidal effects on certain adherent bacteria obviously by a physico-mechanical mechanism. This contact killing is thought to prevent biofilm formation at a very early time point.

We already demonstrated the antibacterial effect of Cicada wing-like surfaces with titanium nano-columns (Ti NC) produced by glancing angle sputter deposition (GLAD) on gram-negative *E.coli* [Sengstock 2014]. However, the underlying mechanism of bacterial destruction on Cicada wing-like topographies, which is suggested to be mechanically induced, has not been proved in detail.

Methods

Cicada wing-like surfaces with Ti NC (high about 480 nm) were fabricated by GLAD on silicon substrates (25 mm²). *E.coli* was allowed to adhere and proliferate on these samples either under optimal cell growth conditions (brain heart infusion medium, BHI) or limited growth conditions (RPMI1640 medium). The dead/living ratio of adherent bacteria was analyzed by staining with BacLight™ and bacterial proliferation was biochemically halted using DL-serine-hydroxamate. Scanning electron microscopy was used for morphological analysis.

Results and Discussion

E.coli adhered to the samples in both different media. Compared to BHI, bacterial growth in RPMI1640 and the dead/living ratio of adherent bacteria were significantly decreased. In addition, when the proliferation was biochemically halted a comparable decrease in the dead/living ratio of adherent bacteria was obtained even in BHI medium.

These results indicate that cell growth of adherent *E.coli*, which is accompanied by cell elongation of the rod structure, is involved in the physico-mechanical killing induced by the Ti NC surface.

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Implant-directed magnetic drug targeting: influence of different implant materials on the accumulation of magnetic nanoporous silica nanoparticles for intended in vivo application.

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Question: Implant-related infections are dreaded complications after implantation of endoprosthesis or osteosynthesis devices. To reach the poorly supplied area and invade the biofilm, effective carrier systems for adequate therapeutics are needed. Crucial point for a most efficient accumulation is the magnetic field strength (H) at the desired location, which could be intensified by implants as weak magnetic sources establishing a field gradient to guide magnetic nanoparticles.

The current study should examine if implant materials with different magnetic properties lead to different accumulation quantities of magnetic nanoporous silica nanoparticles (MNPSNPs).

Method: MNPSNPs consist of Fe₃O₄ cores and a silica shell. Fluorescein-isothiocyanate is covalently attached to the shell. A peristaltic pump was used to establish a closed circuit for 10 minutes (flow velocity 12.2 mm/s) which contains either ferritic, martensitic or titanium control plates within a suspension of MNPSNPs in NaCl (230 µg/ml). During circulation, the plate was positioned between the poles of an electromagnet (H ~ 1.8 T). In an additional set up, the electro magnet was switched off after the 10 min and the pump operated for further 3 min to test remanent properties. Fluid samples were taken and analyzed using a microplate reader in comparison to the origin suspension.

Results: While martensitic plates retained significantly more MNPSNPs than ferritic plates and controls, the amount of accumulated MNPSNPs at ferritic plates did not differ significantly from controls. The anticipated remanent properties of martensitic plates were shown by higher percentage of MNPSNPs after additional circulation time without external magnetic field.

Conclusion: The amount of accumulated MNPSNPs can be influenced by choosing different materials. Since extravasation of MNPSNPs in vivo will occur, the influence of these two materials on the accumulation is important and is currently examined using a mouse model.

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Bone replacement materials for osteoporotic fractures

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Due to demographic change, the need for bone replacement materials for elderly patients is increasing. Osteoporosis is characterized by increased fracture sensitivity and reduced bone mass. Novel bone replacement materials that meet the requirements of osteoporotic fractures were investigated in here. It is described that the increase of osteoblastic proliferation can be increased with strontium (Sr). The ZWBF Aachen has therefore produced β tricalcium phosphate (β -TCP) and hydroxyapatite (HA) by slip casting and introduced Sr into the atomic lattice. Most available materials are superficially coated with Sr, resulting in a "burst release" when implanted. By the integration into the atomic lattice Sr release takes place during the entire resorption of the materials in the defect.

It was revealed that the amount of Sr added to the apatite plays a decisive role in the cytocompatibility with human mesenchymal stroma cells (hMSC). Different Sr compositions were tested: B-TCP with 1.62 wt% Sr, β -TCP with 12 wt% Sr and 80 wt% β -TCP with 18.38 wt% HA and 1.62 wt% Sr. All material were tested in comparison to pure β -TCP which is already established in the clinic. Of all Sr-containing materials, β -TCP with 1.62 wt% Sr performed best. B-TCP with 12 wt% Sr induced necrosis. B-TCP with 1.62 wt% Sr supported the proliferation of MSC better than 80 wt% β -TCP with 18.38 wt% HA and 1.62 wt% Sr. Since bone does not exclusively consist of MSC, a more complex co-culture model of hMSC and endothelial cells was applied to the materials to investigate the effect of Sr on osteogenic differentiation. In addition, the influence of laminar medium flow on the osteogenic differentiation was investigated. For this purpose, the incubation in a bioreactor was compared to the incubation under static conditions. Scanning electron microscopic images revealed an intensive extracellular matrix formation during incubation in the bioreactor. Real-time PCR studies confirmed that results.

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Hydration mechanism of a β -tricalcium phosphate cement (β -TCP) modified with phytic acid (IP6)

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Introduction

Brushite forming biocements have an improved bone regeneration capacity compared to their apatite forming counterparts [1]. Since the rate of crystal growth of brushite is high [2], setting retarders (IP6) have to be applied to increase the setting time to clinically acceptable values of 5-10 min [3].

Materials and Methods

This study aims to investigate in detail the effect of phytic acid as setting retarder in brushite forming cements based on mixtures of β -TCP and orthophosphoric acid (H_3PO_4 , 85 %). For this a mixture of β -TCP with H_3PO_4 , water and different amounts of IP6 (5-20 wt%) were compared to a reference consisting of β -TCP mixed with 5 M $C_6H_8O_7$ solution. The produced pastes were tested in respect to their injectability, rheology during setting and the zeta potential.

Results and Discussion

It could be shown, that the addition of IP6 as low as 5 wt% had an enhancing effect on the injectability and the pastes remained nearly completely injectable even 10 min after mixing of the solid and the liquid phase. A higher amount of added IP6 had no significant effect on the injectability after 5 min, but lead to a better injectability after 10 min. In comparison to the reference the zeta potential was decreased drastically but compared among themselves the IP6 samples showed no huge difference. Adding IP6 increased the initial viscosity due to the formation of chelate complexes consisting of Ca^{2+} ions and the phosphate groups of IP6. This inhibited the typical dissolution/ precipitation reaction and had a retarding effect on the setting process. The setting time could be elongated to an acceptable length by the addition of IP6. The rheology and injectability could also be improved because of chelate complex formation and a decreased zeta potential.

[1] F. Tamimi, et al., *Acta Biomaterialia* 8(2) (2012) 474-487.

[2] C. Oliveira, et al., *Chemical Engineering Research & Design* 85(A12) (2007) 1655-1661.

[3] S. Meininger, et al., *Sci. Rep.* 7 (2017).

Figure 1

Figure 1: Results for injectability (A), zeta potential (B) and rheology depicted as viscosity (C) and dynamic moduli (D)

P30

Dual-setting brushite-silica gel cements – Variations of the monomer precursors and tuning of the setting parameters

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Introduction

Calcium phosphate cements (CPC) in general set via a dissolution–precipitation reaction of the cement raw materials. A further modification by adding monomers to the aqueous cement phase results in the formation of a second interpenetrating network with a strong impact on cement properties.[1] This was recently shown for cement modification with tetraethyl orthosilicate (TEOS) as precursor.[2] Here, this approach is further explored by using silica precursors with a higher density of alkoxy groups to increase network density in the final gel. The precursors were initially hydrolysed under acidic conditions and the combined with a brushite forming cement powder. Due to the increase in pH of the sol during cement setting leads to a simultaneous formation of cement matrix and silica hydrogel.

Materials and Methods

Silica sols were obtained by adding a mixture of precursors ($X = (100-60\%)$ TEOS and $(0-40\%)$ 1,2-Bis(triethoxysilyl)ethane or 1,8-Bis(triethoxysilyl)octane) to water and 0.1 M HCl solution. The influence of the water to alkoxy-group ratio (R) was also analyzed. Composite cements were produced by mixing the sol with cement raw powder, consisting of β -tricalcium phosphate (β -TCP) and anhydrous monocalciumphosphate (MCPA) in an equimolar ratio and the addition of 1 wt% citric acid (retarding agent).

Results

The results demonstrated that increase of mechanical performance by using 20% 1,2-Bis(triethoxysilyl)ethane in the composite compared to the pure TEOS reference (Fig. 1A). The porosity characteristics of the silica–brushite networks showed a bimodal pore size distribution in the set matrices with nanosized pores originating from the silica matrix and μm pores from the cement (Fig. 1B). The latter is thought to have a strong effect on drug release capability by retarding drug diffusion from the cement matrix.

[1] T. Christel et al, *Materials in Medicine*, 2013, 24(3), 573-581.

[2] M. Geffers et al, *Acta Biomaterialia*, 2015, 11, 467-476.

Figure 1

Fig.1 A Compressive strength of pure brushite cement and dual-setting cements with various sol compositions of the cement liquid, **B** Pore size distribution analyzed by Hg porosimetry in a range of 10–10000 nm of silica gel composite.

A novel bioglass lacking hydroxyapatite deposition for connective tissue repair

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Joint cartilage injuries and deep wounds often represent conditions of severely reduced tissue repair affecting resident cells such as articular chondrocytes (ACs), mesenchymal stromal cells (MSCs) or dermal fibroblasts (dFbs). In the past, bioactive glass scaffolds were developed to fill bone defects and also colonized with chondrocytes. However, the hydroxylapatite phase is inappropriate for cartilage and skin regeneration. Hence, the question arose whether cartilage repair, faster wound healing and reliable coverage can be achieved through the development of a completely novel bioglass composition without hydroxylapatite phase. This interdisciplinary project aims to test the properties of a novel bioactive glass scaffold with three representative primary cell types. Scaffolds were colonized with porcine ACs, human dFbs and bone-marrow mesenchymal stromal cells (hMSCs) for 24 h - 35 d. The cell survival in the scaffold was determined with a vitality-assay. For detection of the ECM synthesis, type I and cartilage-specific type II collagen were immunolabeled. In addition, the dFb marker tenascin C and the myofibroblasts marker α -smooth muscle actin were analyzed. Phalloidin-A488 staining was used to depict actin cytoskeleton organization. Scanning electron microscopy provided insights into the ultrastructure and cell-scaffold interactions. Differently configured highly porous bioglass scaffolds allowed the survival, growth of ACs, hMSCs and hFbs. ACs formed cartilage-specific collagen type II and hFbs synthesized type I collagen, tenascin C and α SMA on our newly developed bioglass. Cytoskeleton stainings and SEM revealed numerous focal adhesion points of the cells reflecting an intimate cell-scaffold interaction. This suggests that our modified bioglass is promising for a wide range of applications. In the future the proliferation behavior and synthesis profile of hMSCs for chondrogenic markers will be characterized and quantified including a longer period of time.

P33

Silver doping of the glass-ceramic Bioverit® - bone replacement material with antibacterial properties

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Introduction

Since the 1980s, the glass-ceramic Bioverit® has been used as a bone substitute, mainly in the head and neck area. It is bio-inert, corrosion resistant, and has a high mechanical stability. When used in bacterially contaminated implant sites (middle ear, paranasal sinuses), biofilm formation occurs on the implant surfaces. A possible reduction of this colonization can be achieved by doping the material with silver ions.

Material and methods

Test specimens were made of Bioverit® by CNC milling, and doped in molten salt of AgNO₃/NaNO₃, whereby in the glass phase sodium ions are replaced for silver ions. The microscopic examination of the surface of autoclaved specimens (121°C) was performed with the microscope VHX-6000 (Keyence). Further characterization of the surface and the measurement of penetration depths were carried out with SEM and laser ablation. Eluates of the specimens were analyzed by ICP-OES to determine the release behavior in physiological solution. In addition to cytotoxicity tests (WST1 tests on HeLa cells, elution and colonization tests), the antimicrobial effect was analyzed by tests of bacterial colonization.

Results and discussion

With silver doping, a large proportion of silver ions was successfully brought into the surface of the glass-ceramic Bioverit®. The microscopic examination of the surface and the analysis with SEM and laser ablation showed an inhomogeneous distribution of the silver ions. Based on the cytotoxicity tests, good biocompatibility has been demonstrated for various concentrations of the dopant. First results of microbial colonization tests indicated an inhibitory effect on typical pathogens.

Overall, these first results show a simple way to functionalize the surface of a proven bone replacement material. Further research interests include the realization of a more homogeneous distribution of silver ions and the testing of the antimicrobial effects in a biofilm model.

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Multifunctional Nano- and Macroporous Bioactive Glass-Composites for Bone Regeneration

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Bone replacement after infection or cancer debridement are a critical problem in bone surgery. Therefore, the aim of the presented work is the development of a biodegradable implant material which can combat bacterial infection and support the formation of new bone at the same time.

In a first step nano- and macroporous bioglass scaffold are produced via a sponge template method. A PU sponge is soaked with a precursor solution containing TEOS, as a silica source, ethanol, water, hydrochloric acid and F127 as structure directing agent. Additionally, it is possible to add a calcium or phosphate source. After coating and drying the initial sponge is removed together with the SDA by calcination at 600 °C [1]. For improved mechanical stability and elastic behaviour of the scaffolds these are coated with biodegradable polymers, like poly(trimethylene carbonate) and polyglycerol sebacate. The latter can partly be replaced by a phosphate species to support bone formation. For a further functionalization nanoporous silica nanoparticles can be incorporated into the polymer matrix [2]. The materials are characterized via scanning and transmission electron microscopy, X-ray diffraction, sorption experiments, infrared spectroscopy and compression tests.

The sponge replicas show nanopores of about 5 nm and macropores in the range from micrometre to about 1 millimetre. The developed multifunctional scaffolds are promising candidates for an effective bone replacement material.

References

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- [2] Qiao Z.A., Zhang L., Guo M., Liu Y., Huo Q., *Chem Mater* 21 (2009) 3823.

P35

Calcium and phosphate precursors in hybrid glass scaffolds for bone tissue engineering – incorporation and mechanical properties

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A wide range of different biomaterials is under extensive investigation to create scaffolds for substantial bone loss treatments. This scaffolds material should provide 1) mechanical strength, 2) biodegradability, 3) a porous structure for cells to invade and attach as well as 4) bioactivity to support bone regeneration. We recently presented a hybrid biomaterial from a sol-gel derived glass and 3- or 4-armed macromers. By means of indirect 3D printing, these hybrid materials were processed to remarkably strong macroporous scaffolds [1]. Material's in vitro degradation behaviour and the continuous linear weight loss were analysed over 12 months in a long-term study [2]. In a second step, by addition of 30% Ca^{2+} and 5% phosphate precursors we generated bioactive hybrid glass scaffold [3], but after incubation in aqueous media, its mechanical strength decreased rapidly. In order to improve the mechanical stability and to keep the bioactivity, we systematically reduced Ca^{2+} and varied phosphate content as well as macromeric crosslinker type and amount. A formulation with 10% Ca^{2+} and 10% phosphate as triethylphosphate (TEP) in a sol composition with 30% of functionalized 4-armed pentaerythritol ethoxylate (Mn ~797) is currently considered to be most promising because of its high bioactivity and acceptable mechanical strength. Systematic investigations on the influence of TEP showed that increasing amounts of TEP improved mechanical stability of the scaffold up to a content of 10%. Nonetheless, NMR investigations on the hydrolytic stability of TEP showed missing hydrolysis as a prerequisite for integration into the hybrid glass during sol preparation and processing. Investigations on the role of TEP during scaffold fabrication are ongoing as well as Ca^{2+} release studies from the final scaffolds.

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[2] C.Kascholke at al.,Acta Biomaterialia 63(2017):336-349

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Figure 1

Comparison of computational fluid dynamics methods for characterisation of permeability in tissue engineered ceramic scaffolds

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Regeneration of bone in critical size defects is a challenging task due to different, often contradictory, requirements imposed on these materials. Utilising 3D scaffolds as tissue templates has proven a promising approach. Ceramic materials have gained increasing use in bone tissue engineering due to their biocompatibility and similar chemical composition as bone. Particular bioactive glass (BG), is still one of the most important biomaterials for bone defect repair. Boccaccini and co-workers have characterised BG scaffolds in terms of compressive strength and effective diffusivity. However, no assessment of scaffold permeability was performed.

This work investigates performance and accuracy of a variety of computational techniques used to simulated fluid flow in porous materials. These techniques include the (i) finite volume method and (ii) smooth particle hydrodynamics methods. Based on pore-scale simulations we then calculate the permeability of the BG 3D scaffolds. Permeability is directly linked to microstructural flow characteristics of scaffolds and may serve as a surrogate measure for blood vessel ingrowth. Scaffolds exhibiting low permeability may not be suitable for bridging of large bone defects due to hindrance of blood vessel ingrowth. The present work compares different numerical techniques commonly used to solve the Stoke"s equation applied on the pore scale in terms of computational efficiency (i.e. computation time) and numerical accuracy.

Performing micro computed tomography (microCT) analyses of the generated BG 3D scaffolds will provide essential information on pore microstructure. We will then import these microstructures into different numerical software packages for calculation of the pore scale fluid flow. Volume averaging of the velocity fields and pressure gradients allows to calculate the scaffold permeability.

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Biomaterial degradation and ion release in flowing medium and under static incubation conditions

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Degradation and release of cations from degradable bone substitute materials was investigated, with focus on the influence of degradation regime parameters. For this purpose, a flow chamber was developed, which allowed degradation and bioactivity study under dynamic liquid flow conditions. Results were compared with classical incubation experiments with regimes of static and regularly changed medium.

Materials/Methods

Silica/Collagen xerogels modified with a mineral phase: commercial hydroxyapatite, strontium phosphate, and custom-made calcium/strontium hydrogen phosphates with different ratios of Ca/Sr (= 10/0; 5:5; 3:7; 0/10) were produced. The monolithic specimens were characterized by XRD and SEM before and after incubation alpha-minimum essential medium with low (0.5 mM) and standard (2.4 mM) calcium ion concentration. Incubation regimes applied were without and with medium change twice a week as well as under constant medium flow. In all experiments, the same summary media volume was used per 7 days. Ion concentrations of cations and pH was measured as well as the change in specimen masses.

Results/Discussion

Initially all materials in this study caused a decrease of calcium in the incubation media. This is due to the high bioactivity of the silica matrix. Interestingly, the initial decrease was enhanced by hydroxyapatite and commercial strontium phosphates. In contrast, custom-made calcium/strontium hydrogen phosphate (especially Ca/Sr = 5:5 and 3:7) were able to cause an increase in ion concentration with time by cation release.

Interestingly, bioactivity of materials was influenced to the same extent by incorporated mineral phases and the media regimes. More information about material degradation was gained from comparison of static and flow regime than from comparison of static and regularly changed medium. Therefore, *in vitro* investigations of biomaterials degradation should incorporate media flow, to stepwise close the gap to the *in vivo* situation.

Figure 1

Figure 2

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P39

Loading and release experiments in PBS for drug delivery applications: Influence on porosity and stability of nanoporous (organo)silica nanoparticles

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Nanoporous nanoparticles from silica nanoparticles or periodic mesoporous organosilica are promising materials for drug delivery applications due to their high permanent porosity, chemical variability and biocompatibility [1]. For this application, it must be taken into account that these materials degrade in aqueous media, a process which is further influenced by external parameters (e.g. release medium) [2]. It is expected that porosity changes during release, but there are only few studies with regard to this aspect. Therefore, this work focusses on the synthesis and characterization of nanoporous silica nanoparticles (NPSNPs; Abb. 1a) [3] nanoporous organosilica nanoparticles (NPOSNPs; Abb. 1b) and core-shell-nanoparticles consisting of a NPSNP core and a NPOSNP shell (Abb. 1c) [4], and concentrates on the changes of their physisorption properties during a release study.

For this study, these nanoparticles were loaded with the dye methylene blue, as reference nanoparticles were treated in the same way without the guest molecule. With these loaded particles, release experiments in phosphate buffered saline (PBS) were performed and after certain time intervals the release was stopped to characterize the influence of the release conditions on the particles on BET surface area, total pore volumes and pore size distributions at different times up to 10 weeks. Generally, a continuous decrease of the BET surface area and the total pore volume were observed.

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Abb. 1: a) NPSNP; b) NPOSNP; c) core-shell nanoparticles.

Figure 1

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Mesoporous silica nanocarrier with a pH-responsive gatekeeper system for drug delivery

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Poor survival rates for head and neck squamous cell carcinoma stagnate since decades due to limited therapeutic options, frequent recurrences and metastasis. Therefore, it is crucial to develop new therapeutic strategies. For example, targeted drug carriers such as dendritic mesoporous silica nanoparticles (MSN) are highly promising. MSN possess a unique structure with an adaptable particle and pore size, can be easily functionalized on the surface and simply loaded with various drugs. Most importantly, MSN are highly biocompatible. MSN are evaluated as nanocarrier to head and neck cancer cells. An innovative gatekeeper system consisting of ferrocene enclosed by β -cyclodextrin is linked to the MSNs' surface to control the release of active agents. The amide bond between the ferrocene and the amino-functionalized MSN is hydrolyzed in an acidic environment and the cargo will be liberated inside of cancer cells. MSNs showed uniform size and morphology. Cy-5 labeled MSNs were taken up by the endolysosomal system in tumor cells which was observed via confocal microscopy. MSN showed good biocompatibility up to 100 $\mu\text{g}/\text{mL}$ MSN in various cancer cell lines and mouse embryonic fibroblasts. Also, a retarded release of the model drug Doxorubicin could be observed via toxicity analysis and evaluation of $\gamma\text{H2A.X}$ foci which indicate DNA double-strand breaks.

So, mesoporous silica nanocarriers with a pH-responsive gatekeeper system are suited for drug delivery targeting cancer. Cancer cell specific uptake could be enhanced by coupling MSN with an epidermal growth factor receptor antibody.

P41

An enteric coating system for the oral administration of functional nanoparticles into the colon

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Calcium phosphate nanoparticles are biocompatible, biodegradable, easy to stabilize and because of their small size, can be taken up by cells. Simultaneously, they can be loaded with functional molecules and transported across the cell membrane [1].

A targeted drug delivery into the colon is highly desirable to treat diseases like Crohn's disease, and also for a systemic delivery of protein and peptide drugs [2, 3]. A colon-specific drug delivery system should be capable to protect the drug after oral administration on its way through the stomach to the colon. A new enteric coating dispersed system was developed to prevent a dissolution of the functional nanoparticles in the acidic environment of the stomach.

In order to achieve a time- and site-specific release, gelatine capsules containing the freeze-dried functional nanoparticles were covered by dispersing chitosan in the methacrylic acid copolymer Eudragit® L100. This pH-sensitive polymer is insoluble in gastric acid but dissolved at slightly basic pH (as in the colon). By adjusting the coating composition, we were able to control the drug release in a time- and site-specific way. The nanoparticle release rate was controlled by changing the volume ratios of Eudragit and chitosan.

Both positively and negatively charged calcium phosphate-based nanoparticles were prepared and characterized by dynamic light scattering (DLS), scanning electron microscopy (SEM), and atomic absorption spectrometry (AAS). The release rate of the freeze-dried nanoparticles (together with the cryoprotectant trehalose) from the capsules was analysed by ultraviolet-visible spectrophotometry (UV/Vis) and atomic absorption spectrometry (AAS). In *in vitro*-experiments, the particles were shown to survive the acidic environment of the stomach (pH 1) inside the coated capsules. An increase in pH to 7.4 led to a rapid release of dispersed nanoparticles out of the dissolved capsule after about 30 min.

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P42

Functionalization of Nanoporous Titania Coatings for Dental Application

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Although tooth implants are a common treatment in nowadays dentistry, peri-implantitis is a major health issue. Bacteria may form biofilms on the implant surfaces, due to late infections, causing high implant failure rates (>10%) [1, 2]. To prevent and cure these infections antibacterial surfaces are required.

In our work, we are going to introduce a stimuli-response drug delivery system to the implant surface. Therefore, a nanoporous titania film is chemically deposited onto the implant surface and loaded with antibiotics. The diffusion out of the pores is blocked due to the surface modification with a biopolymer. In case of an infection the pH value decreases, causing a positive charge at the polymer chains and repulsion between those, leading to an opening of the pores.

The system was already successfully synthesized in our group on silica nanoparticles [3]. The first step to transfer the system to a titania surface was to establish nanoporous titania films, utilizing an adjusted cathodic electrodeposition method of Hu, Huang and co-workers [4]. To attach the pore blocking polymer the titania surface has to be modified first. This can be achieved using phosphonic acids, which are suitable because of their strong chemical bonds to titania [5]. Within our latest work we were able to successfully attach different phosphonic acids to our electrodeposited titania surfaces. The modified and unmodified nanoporous titania films were examined by scanning electron microscopy (SEM), laser Raman microscopy (LRM), X-ray photoelectron spectroscopy (XPS), zeta-potential measurements and contact angle measurements (CA).

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P43

Effect of different thin ceramic coatings on the biomechanical stability of femoral implants

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Background

Modern implant coatings can improve osteointegration of orthopedic implants and reduce implant loosening. The most effective materials and optimal characteristics are currently unknown. This study aims to assess the effect of thin coatings consisting of β -tricalcium phosphate (β -TCP), $\text{Ca}_2\text{KNa}(\text{PO}_4)_2$ (GB14), hydroxyapatite (HA) and Bioglass on shear strength of femoral implants at different points of time.

Methods

We surgically placed 146 titanium bone implants with 20 μm thick coatings of β -TCP, GB14, HA or Bioglass bilaterally into the distal femoral metaphysis of 73 New Zealand White rabbits. The rabbits were allowed unrestricted weight bearing. The femora were harvested 2, 4, 6, 12 or 24 weeks after implantation. Capacity to withstand shear forces was assessed through push-out testing with the Universalprüfmaschine Zwick Z020 under visual control. Afterwards, microscopy was performed to determine structural integrity of the coating.

Results

Implants coated with HA achieved average shear strength of 1.92, 2.42, 2.39, 3.44 and 2.84 Megapascal (MPa) after 2, 4, 6, 12 and 24 weeks, respectively. Those with a β -TCP coating reached 3.27, 3.87, 3.24, 3.91 and 3.30 MPa. GB14-coated implants arrived at 0.94, 3.11, 3.62, 3.66 and 5.47 MPa. Implants coated with Bioglass achieved 0.35, 0.17, 0.29, 1.04 and 2.26 MPa. At 2, 4 and 12 weeks, β -TCP coating lead to the greatest shear strength. GB14-coated implants saw continuous increases in shear strength and reached the highest overall shear strength of all implants at 24 weeks. Implants with HA coating performed similar to GB14-coated implants except for lower shear strength at 24 weeks.

Conclusions

The greatest average shear strength in the earlier stages between 2 and 12 weeks was achieved by implants coated with β -TCP. At the final 24 week mark GB14-coated implants reached the highest shear strength of all implants. Bioglass-coated implants saw the lowest shear strength at all harvesting times.

P44

A genetically active coating of cochlear implant electrodes with calcium phosphate nanoparticle

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Severe hearing problems such as impaired hearing or deafness are frequent diseases. These can be caused by damaged and degenerated hair cells in the inner ear, resulting in an atrophy of spiral ganglion cells (SGC). To regain the ability to hear, cochlear implants are a common treatment. The possible regain of hearing depends on the duration and the intensity of the degeneration of the cells inside the inner ear. To enhance regeneration and formation of spiral ganglion cells, a genetically active coating of the implants electrodes was developed. To this end, a biocompatible coating consisting of DNA-functionalised calcium phosphate nanoparticles was applied to a model substrate by a layer-by-layer technique.

The particles were prepared in a precipitation method by mixing calcium nitrate and diammonium hydrogen phosphate under stirring and stabilised by protein-encoding plasmid DNA (pDNA) as described earlier [1]. The nanoparticles were characterised by dynamic light scattering (DLS), scanning electron microscopy (SEM), and atom absorption spectroscopy (AAS). The pDNA concentration was determined by UV-VIS.

As a model substrate, glass cover slips were coated in a layer-by-layer method with a solution of poly-L-lysine hydrobromide (PLL) and a dispersion of the DNA-loaded calcium phosphate nanoparticles. The coating was completed with a final layer of PLL and characterised by AAS and SEM [2].

Cell transfection experiments on HeLa cells with pcDNA3.3-eGFP loaded nanoparticles confirmed the applicability of our approach. Furthermore, cell experiments with primary rat cochlear cells showed the uptake of nanoparticles after 24 h.

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P45

NANOPARTICLE-MEDIATED TRANSFECTION OF EUKARYOTIC CELLS IN 2D AND 3D CELL CULTURE MODELS

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Nucleic acid delivery enables to modify gene expression in cells. Calcium phosphate nanoparticles may serve as carriers of nucleic acids into cells. They are biocompatible, biodegradable, and are readily taken up by eukaryotic cells [1]. 2D and 3D cell culture models have a wide range of *in vitro* applications. When compared to a monolayer 2D model, a spatial 3D model better reflects the cytoarchitecture of a tissue and may serve as bridge between *in vitro* and *in vivo* studies [2,3].

Our aim was to synthesize and characterize calcium phosphate nanoparticles, loaded with mCherry-encoding DNA, and to evaluate the efficiency of nanoparticle-mediated cell transfection in 2D and 3D culture models.

Polyethyleneimine-stabilized calcium phosphate nanoparticles were loaded with mCherry-encoding plasmid DNA and coated with a silica shell. The nanoparticles were purified, characterized by DLS, AAS and SEM, and used to transfect mono- and co-cultures of HeLa-EGFP and MG-63 cells grown in 2D and 3D culture models. The efficiency of transfection was determined by fluorescence microscopy.

Calcium phosphate nanoparticles were spherical with an average size of 105 nm and a zeta-potential of +28 mV. In 2D mono-cultures, the transfection efficiency of HeLa-EGFP cells was higher than in co-cultures of HeLa-EGFP/MG-63 cells. For MG-63 cells, the opposite effect was observed. In 3D mono-cultures, the transfection efficiency of HeLa-EGFP cells was higher than that of MG-63 cells. The transfection efficiency in 3D co-cultures was generally low.

Calcium phosphate nanoparticles can serve as carriers of nucleic acids into cells in 2D and 3D culture models. They have the potential to be applied in gene therapy.

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P46

Design of inorganic nanocarriers for siRNA delivery

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RNA interference (RNAi) is a gene regulatory mechanism, in which small interfering RNA (siRNA) initiates the cleavage of target mRNA strands. Since its discovery, RNAi has emerged as promising technology for influencing gene expressions. Nevertheless, the delivery of siRNA into a cell is still challenging due to poor intracellular uptake, limited stability of siRNA molecules and cell toxicity of current transfection agents [1,2,3].

Our approach is to use nanoporous silica nanopartilces (NPSNPs) as delivery platform for siRNA. In the past decade, NPSNPs have emerged as promising basis for designing tailored delivery systems for a variety of biomolecules (small molecule drugs, proteins or nucleic acids). Among other positive features these materials exhibit high surface areas, high porosities and reactive silanol groups at the surface, which can easily be modified. Moreover, NPSNPs are biocompatible and biodegradable [2,4].

NPSNPs were prepared via sol-gel process using polystyrene as structure directing agent to build up the porous system [5]. After template removal the particle surface was modified with different trialkoxysilanes by post-grafting reactions to control siRNA uptake and release. The immobilized and released amounts were quantified via UV-Vis and fluorescence spectroscopy. The siRNA loaded particles were capped with different agents to enhance cell uptake.

The synthesized NPSNPs were 70 nm in size and had an inner surface area of about 830 m²g⁻¹ and a pore diameter of 7 nm. By modifying the surface, the release behavior could be adjusted from burst to a linear release. Cell uptake investigations showed siRNA located near cell nucleus. The biological efficacy will be tested in further gene knockdown experiments.

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P47

Immobilized and free standing 3D fibrinogen nanofiber scaffolds

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Fibrinogen (Fg) is a plasma protein, which is involved in blood clotting and wound healing [1]. Because of this crucial role Fg nanofibers are highly attractive biomaterials. Previously, Fg nanofibers have been prepared *in vitro* using, for example, electrospinning [2], acidic buffers [3] or hydrophobic surfaces [4, 5].

Here, we show for the first time that Fg nanofibers can be prepared by salt-induced self assembly. Different buffers including phosphate buffered saline (PBS) and other Na- or K-based buffers induced fiber formation with diameters of 100 to 200 nm (Fig. 1). Fibrillogenesis was induced on different substrates including glass and gold with a minimum protein concentration of 2 mg/ml. The optimum pH range to prepare dense nanofiber assemblies was between pH 7 and 9. With increasing PBS concentration we could assemble Fg into 3D-nanofibrous scaffolds with dimensions in the centimeter range and a thickness of several micrometers.

When nanofibrous Fg scaffolds on glass were crosslinked in formaldehyde vapor and washed in aqueous buffer we could induce controlled detachment and obtained free-standing Fg patches. The nanofibrous scaffold morphology was maintained during this detachment procedure. However, when the glass surface was modified with an amino silane prior to Fg self assembly the nanofibers stayed immobilized on the surface after fixation and washing. Hence, our novel self assembly procedure facilitates the simple and tailored fabrication of macroscopic Fg nanofiber scaffolds. Since either free-floating or immobilized 3D-scaffolds can be prepared "on demand" our novel Fg scaffolds are promising biomaterials for future cell culture and wound healing applications.

Fig. 1: Fg nanofiber scaffolds on glass were detached upon washing.

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Figure 1

P48

3D bioplotting of a composite material for adjusted bone healing

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Introduction

For bone regeneration, composite materials of ceramics in a polymer matrix have shown promising results. They have proven high significance in areas like cell adhesion and proliferation, mechanical properties, osteogenic differentiation and mineralization^{1,2}.

Objectives

The aim of this study is the 3D bioplotting of a three layered hybrid structure based on alginate³ for the reconstruction of critical cranial bone defects in surrounded soft tissue. Each layer has its own function and composition, in order to ease bone growth.

Materials & Methods

The first layer is built of dense pre-crosslinked alginate (Alg), second is plotted on top of the dense one and consists of hydroxyapatite (HA) as a filler component in an Alg-matrix. Challenges are to generate it with pores in *x*, *y* and *z*-direction and to offer osteogenic behavior. Also gelatin was added to the composite to improve cell adhesion. On top, a dense alginate layer (s. first layer) is again plotted. Both have the task to prevent the ingrowth of cells, to guarantee an ingrowth only in the middle layer. The whole construct is analyzed using microscopy and the technique of μ -Computed-Tomography (μ -CT). Furthermore, *in-vitro* cell tests (MC3T3-E1) are realized

Results

With optimized plotting procedure the layers are plotted above each other without converge of them. Light microscopy and μ -CT analysis show distinguishable interface and interconnected pore systems of the composite layer. *In-vitro* cell tests (MC3T3-E1) show already promising results.

Conclusion

All tests revealed, that first milestones are taken and auspicious results were achieved. However, there are still plenty of improvements and analysis to be done to fulfill the aim for a clinically working bone healing construct.

Acknowledgement

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P49

Extracellular matrix as smart biomaterial for adipose-derived stem cell differentiation

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The extracellular matrix (ECM) of a tissue provides not only physical support but also initiates crucial biochemical signals that influence cell adhesion, morphology, proliferation and differentiation. The composition and physical characteristics of the ECM differs between different tissues and different stages of cell differentiation. Since cell produced ECM is closest as possible to the native surrounding of the cell in tissue it represents a promising smart biomaterial for tissue engineering. The present study investigates the influence of ECM from adipose-derived stem cells (ASC) of different stages of adipogenic differentiation on ASCs regarding adhesion, cell morphology, proliferation and adipogenic differentiation. ASCs were isolated from tissue samples from donors undergoing plastic surgery by enzymatic digestion. To generate adipogenic ECM cells were cultured in adipogenic differentiation medium for one week. After lysis of the cells the remaining ECM was re-seeded with ASCs of the same donor. As controls cells were seeded on undifferentiated ECM and native TCPS. Adhesion of the re-seeded cells was examined by light microscopy. Proliferation and viability assays were performed to investigate the influence of differentiated and undifferentiated ECM on ASC. To determine the influence of ECM on adipogenic differentiation western blot analysis and ELISA of early and late adipogenic transcription factors were performed. Light microscopy revealed that adhesion on ECM was enhanced compared to native TCPS. Proliferation and viability assays revealed no significant differences between cells cultured on ECM and TCPS. Regarding adipogenic differentiation it can be overserved that culture of ASCs on differentiated and undifferentiated ECM influences expression of early and late adipogenic transcription factors compared to TCPS. Further investigation should include determination of adipokine release of ASCs during culture on ECM.

P51

Plant virus nanoparticles for supported neural stem cell migration in vitro

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Plant virus nanoparticles have been considered as biomaterials due to their low immunogenicity and simple genetic manipulation of their amino acid sequence presented on the outer surface. They are available in varying shapes and sizes, can be purified from infected plants, and have been manipulated for metal binding on electrodes, vaccine or drug delivery systems. Recent studies also provided the opportunity employing them in regenerative medicine as additive to functionalize surfaces or hydrogels for stem cell culture. During mammalian central nervous system development, neural precursor cells produce pools of multipotent and more restricted neural progenitor cells, which then proliferate, migrate and further differentiate into neurons and glial cells. We have developed an in vitro assay based on this neuroprogenitor cells mimicking neural development to investigate developmental neurotoxicity of chemicals. For cell migration, cells need laminin which expresses Integrin binding motifs RGD and IKVAV. Therefore, we have generated and purified plant viral nanoparticles presenting varying RGD and IKVAV amino acid sequences on the surface of potato virus X (PVX), a flexible rod-shaped plant virus of approximately 500 nm in length. We confirmed cytocompatibility to neuroprogenitor cells by mitochondrial activity and membrane integrity. PVX-RGD and -IKVAV presenting nanoparticles both supported cell migration on 2D surfaces. In addition, neuroprogenitor cells were able to differentiate into astrocytes and neurons visualized by immunostainings of respective markers and subsequent analysis by fluorescence microscopy. To conclude, plant viral nanoparticles are promising candidates to functionalize material surfaces and to stimulate neural cells. Future experiments aim at their application in 3D hydrogel-based in vitro models to be used for toxicity assessment of compounds.

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Bacterial nanocellulose containing frankincense extract as tailor-made anti-inflammatory wound dressing

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Question

In the expanding field of biomaterials bacterial nanocellulose (BNC) has attracted much attention due to its outstanding physicochemical characteristics and unique three-dimensional network of nanosized fibers [1]. In our studies BNC was loaded with natural lipophilic molecules with high anti-inflammatory capacity such as triterpenic acids of frankincense. Since it is still challenging to load the highly hydrophilic BNC with lipophilic compounds, the focus of this study was to evaluate the loading efficiency and the release behavior of frankincense extract. The influence of loading techniques and formulation approaches was examined.

Methods

BNC was synthesized by *Komagataeibacter xylinus* at 28 °C in 24-well plates for 14 days or in trays for seven days. Loading of frankincense extracts was performed by absorption or reswelling methods and physicochemical characteristics, loading efficiency and release behavior were investigated using different additives. To determine the penetration ability of the different frankincense formulations into the skin, tape stripping experiments were performed using porcine skin.

Results

By utilizing additional excipients (poly(ethylene glycol), Poloxamers or nanoemulsions) and several loading strategies, different physicochemical characteristics and loading efficiencies of the BNC were obtained. Release profiles were also modified by these approaches. Moreover, skin penetration studies demonstrated that frankincense could be found in deeper layers of the porcine skin.

Conclusion

By varying the loading technique, excipients and the formulation strategy we were able to realize different release profiles of frankincense to develop an innovative tailor-made dressing for chronically inflamed wounds.

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P53

Integration of a Collagen Barrier into an Embroidered Three-Domain ACL-Implant to Avoid Cell Migration *in vitro*

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Introduction

Knee injuries accompanied by ligament rupture prevalently affect the anterior cruciate ligament (ACL). A surgical approach to reconstruct the joint function could be the application of a tissue engineered entity, including the three tissue domains bone, enthesis and ligament. The entity is based on a three-dimensional embroidered scaffold, which is bio-mechanically and bio-functionally adapted and contains a co-culture of the different tissue-specific cell types [1, 2].

Objectives

To avoid commingling of the cells ahead of time, the domains are to be isolated by a barrier composed of a collagen thread, which is integrated into the structure by embroidery technology.

Materials and Methods

Samples were embroidered with a commercial polypropylene thread performing different alignment of the fixation stitch and layers of collagen threads (Fig. 1 A, B). The precision of the deposition was rated on polished specimens (Fig.1, C). Diffusivity of the barrier was determined by applying FITC-Dextran with different molecular weights (40, 150, 250, 500 kDa) in a self-made diffusion chamber system [3]. Cell migration was monitored under static cultivation with cell spheroids.

Results

A precise deposition of the collagen thread could be achieved by aligning the fixation stitch in a sinus wave. A barrier function could be shown by diffusivity (Fig. 2). A dependency on molecular weight and the number of collagen layers as well as a restrained cell migration could be assumed.

Conclusion

The embroidered collagen barrier might be a feasible insertion to avoid cell migration during *in vitro* co-cultivation

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Figure 1

Figure 1: (A) scaffold with collagen barrier, (B) arrangement of collagen threads and (C) polished specimen in cross section.

Figure 2

Figure 2: Diffusivity of scaffolds a) without and with b) one layer or c) two layers of collagen.

P54

ADA-GEL Hydrogels for Induced Pluripotent Stem Cell Derived Neural 3D Cell Culture

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In vitro neurotoxicological screening tests for safety assessment of compounds and chemicals should be of human origin, standardized, reproducible, well characterized and allowing a medium-throughput. Human induced pluripotent stem cell (hiPSC) differentiate in all three germ layers, can be personalized, and avoid ethical concerns. Three-dimensional (3D) bioprinting may realize these requirements by producing organ-like neural structures containing neurons and glia cells. However, 3D bioprinting of functional neuronal networks is challenging due to the complexity of brain tissues and tailored bioinks providing the optimal neural cell environment are needed. Therefore, in this study, we aim at the development of a neural-cell compatible bioink, which allows hiPSCs derived neural progenitor cells (NPCs), neurons and glia cells to survive and form 3D electronically active neuronal networks. We hypothesized that alginate di-aldehyde (ADA) gelatin (GEL) crosslinked hydrogel is a suitable biomaterial to maintain cell viability and allows for the differentiation into the individual neural cell types. For attachment and cell migration, laminin was included within the ADA-GEL preparation. The porosity of the resulting hydrogels was visualized by scanning electron microscopy (SEM). The activity of hiPSC and hiPSC-derived cells within cell-laden hydrogels was followed over time periods of 28 days. Mitochondrial activity and membrane permeability were investigated to confirm cell viability and cytotoxicity, respectively. The cell morphology and cell-material-interactions were visualized by SEM, immunostainings and subsequent analysis applying fluorescence microscopy. ADA-GEL is a promising bioink for neural tissue engineering. Current and future work aims to further establish this cell-compatible and printable material for standardized and reproducible 3D bioprinted neuronal networks for neurotoxicity evaluation.

P55

Biogenic hydrogels for tissue engineering of cartilage

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In situ cross-linking hydrogels are useful for minimal invasive treatment of tissue defects. The project focused on the hypothesis that semisynthetic sulfated and oxidized cellulose derivatives can be used for the generation of hydrogels with tuneable mechanical properties, degradation behaviour and release of growth factors for tissue engineering of cartilage.

Cellulose was sulphated first via direct sulfation and acetosulfation, followed by a Malaprade oxidation. The obtained oxidized cellulose sulfates (ox-CS) were quantified by RAMAN and 1H-NMR spectroscopy.

The ox-CS derivatives had different degrees of sulfation from 0.7 to 1.5 and oxidation degrees from 0.1 to 0.4. The biocompatibility of these derivatives was analysed *in vitro* studying the metabolic activity and vital staining of mouse fibroblasts exposed to different concentrations of ox-CS. The results show that all CS were non-toxic at lower concentrations (0,5 mg/ml), but with rising oxidation degree and concentrations, toxic effects were observed particularly for highly oxidized CS. By contrast, carboxymethyl chitosan, which was later used as second component for gel formation was not toxic even at highest concentration of 10 mg/ml.

When ox-CS with carboxymethyl chitosan hydrogels were formed within a few minutes. The hydrogels were stable for more than 14 days under cell culture conditions. First studies with encapsulated mouse fibroblasts and adipose-derived stem cells (ADSC) showed maintenance viability over a period of 7 days. In ongoing studies, the ADSCs encapsulated in the hydrogels are differentiated up to 21 days with soluble and immobilized TGF- β 3 to analyse the bioactivity of hydrogels regarding the chondrogenic differentiation of cells.

We conclude that hydrogels composed of semisynthetic oxidized CS might be suitable as building blocks for *in situ* cross-linking hydrogels for tissue engineering of cartilage.

P56

Sulfated Oligo-Hyaluronans in Acrylated Hyaluronan/Collagen Based Hydrogels

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Introduction

Collagen type I and glycosaminoglycans are major components of the extracellular matrix of skin and bone. Thus, they are suitable compounds for functional biomaterials targeting these two tissues. Hyaluronan (HA) based hydrogels have a low immunogenic potential and the inclusion of chemically sulfated HA derivatives (sHA) enables binding and release of mediator proteins involved in healing processes, e.g. TGF- β 1, VEGF [1, 2]. Previously, photo-crosslinked acrylated HA (HA-AC)/collagen based hydrogels including polymeric sHA were shown to tune protein binding and release behavior as well as cellular response [3]. In this study, sulfated acrylated oligo-hyaluronans (oligo-sHA-AC) have been included in hydrogels in comparison to polymeric sHA-AC.

Materials

HA/collagen based hydrogels were prepared by photo-crosslinking as previously reported [3]. The *in vitro* fibrillogenesis of collagen was done in the absence of GAGs, followed by photo-crosslinking of HA-AC, oligo-sHA-AC or sHA-AC and freeze-drying. Resulting gels were characterized in terms of swelling properties, surface morphology, composition and stability as well as binding and release of a relevant protein.

Results

The hydrogel constituents influenced the water content, loss of volume and swelling ratio. While the HA-AC content was rather stable over 8 days in PBS, the sulfated derivatives were released to a greater extent. HA sulfation demonstrated an impact on protein binding and release.

Conclusion

Sulfated HA derivatives could be included and lead to stable hydrogels. The findings on protein binding and release highlight the potential of these gels to influence healing processes in bone and skin.

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P58

Physico-Chemical Properties of Gelatin Type A and Type B Methacryloyls are Dependent on their Raw Materials

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Gelatin type A (G_A) and type B (G_B) are utilized for synthesis of gelatin methacryloyl (GM) acetyl (GMA), which are investigated for biomaterials [1]. However, the impact of gelatin raw material on resulting polymer and material properties beyond the modification degree was rarely investigated, so far. In this study, we compared five chemically modified $G_{A/B}$ derivatives with regard to their physico-chemical properties.

Preparation of $G_{A/B}M(A)$ and determination of degree of methacryloylation (DM) and acetylation (DA) was done following the procedure reported by Claaßen *et al.* [2]. For physico-chemical characterization we determined the isoelectric points (IEP), molecular weight distribution, viscosity, gelation and melting temperature of GM(A) solutions as well as storage moduli (G') of respective hydrogels.

DM and DA increased with increasing utilized molar excess of anhydride, consistent with previous reports [2,3] and were indistinguishable for G_A and G_B . Differences in size exclusion chromatography elugrams were assigned to different hydrophilicity of GM(A)s. The IEP of G_AM was generally higher compared to G_BM . Dynamic viscosities, gelation and melting temperature decreased with increasing degree of modification. Viscosities were indistinguishable for all highly-modified gelatins, see Figure 1. Low-modified G_AM showed lower viscosity compared to G_BM derivatives, which was traced back to different standard viscosities of the raw materials, similar results were observed for G' of GM(A) hydrogels [4].

Material properties of GM(A)s are not just determined by the overall modification degree (DMA) but also on the raw material. The higher the DMA the lower the impact of raw material properties.

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P59

Establishment of Scaffolds with different stiffness for testing the influence of the rigidity on the metabolism of drugs

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Introduction

Drug-Induced hepatotoxicity is the leading cause of acute liver failure drugs. Because the prediction of such adverse events is often not possible in animal studies due the species differences, the pharmaceutical industry is interested in more reliable and predictive in vitro models. In the last years, special focus has been placed on the development of new liver cell culture models, primarily 3D cell cultures. Despite all the progress that has been made, there is no in vitro 3D cell culture system that reliably predicts how drugs will be metabolized during liver diseases. Clinical studies have clearly shown that tissue stiffness has an important effect on drug metabolism (pharmacodynamic and kinetic) and will negatively influence the metabolism of given drug. Aim of this study was to establish scaffolds with different stiffness and testing their metabolic capacity.

Materials

Cryogels with differential stiffness which reflects the stiffness of a healthy, and a cirrhotic liver were created by radical polymerization of 2-hydroxyethyl methacrylate and bis-acrylamide. The scaffolds were characterized (e.g. pore size, stiffness), HepG2 cells were seeded and main hepatic functions were measured.

Results

The developed scaffolds have a stiffness of 2.9 (healthy) and 16.6 (fibrotic) kPa. The higher stiffness is related to a lower porosity and permeability compared to the "healthy" liver scaffold which is similar to the clinical situation. Phase I/II drug metabolizing activity and the urea production of cells is higher in both scaffolds compared to 2D, whereby the "healthy" liver scaffold shows the highest activity.

Conclusions

We have established a 3D cultivation system which resembles the metabolism of a diseased and healthy liver. Thus, the cultivation system presented here could be an ideal tool in the evaluation of drugs during disturbed liver function.

P60

Injectable oligomer-cross-linked chitosan nanocomposite hydrogels as potential regenerative and biomedical material

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Introduction

Novel nanocomposite hydrogels based on natural polymers with embedded nanoparticles (NP) are versatile drug delivery matrices when effectively cross-linked in a controlled and cytocompatible manner. Anhydride group-bearing oligomers developed in our group have been successfully used for the formation of hydrogels with different gelatin fractions.

Objectives

In this project, we aim at the development and characterization of injectable chitosan nanocomposite hydrogel (NC) by combination of newly developed protein NP, as reinforcing fillers and delivery devices, and oligomer cross-linked chitosan as gel matrix.

Materials & methods

OPNMA {oligo(PEDAS-co-NiPAAm-co-MA)}, oPDMA {oligo(PEDAS-co-DAAM-co-MA)} and oPMoMA {oligo(PEDAS-co-AMO-co-MA)} of different compositions have been synthesized by free radical copolymerization. Albumin-NP (ANP) and gelatin-NP (GNP) have been fabricated by desolvation, cross-linked by addition of oligomer solutions (oPNMA and oPDMA) and lyophilized. Lyophilized NP were dispersed in chitosan solution and subsequently cross-linked by addition of oligomer solution in DMSO. The resulting composites were rheologically characterized. Cross-linking density was evaluated to characterize the networks.

Results

ANP and GNP size distribution was assessed by dynamic light scattering and found reproducible (180-300nm). Surface charge of NP was analyzed as a function of environmental pH and was controlled by constituent protein. A protocol for oligomer crosslinking of chitosan solutions was established. NC showed higher elastic modulus and lower degrees of swelling as compared with NP-free controls.

Conclusion

Oligomer-cross-linked chitosan/protein-NP composites were successfully formulated with superior mechanical properties. The NC materials will be further investigated for regenerative applications.

Figure 1

P61

Novel Biocompatible Polymerized Ionic Liquids (PILs)-based Hydrogels as Innovative Drug Carriers in Controllable and Individualized Dosage Forms

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Due to the growing demand for a controlled release of drugs in the pharmaceutical industry, there has been renewed interest in the development of novel and reliable drug-delivery systems. In terms of this challenging task, the literature considers various approaches, including the embedding of drugs in hydrogels. Such innovative systems enable the "smart" delivery of bioactive molecules from drugs. The disadvantages of already existing intelligent hydrogels appear in their low mechanical stability, limited and not completely reversible swelling capacity and slow response to external stimuli. To improve these properties, we synthesize, chemically modify and optimize novel (PILs)-based hydrogels as drug carriers. This research aims to achieve selective drug release from hydrogels over a defined time period and at a specific location in order to control the duration of the action while minimizing undesired effects outside the site of action.

The swelling property of hydrogels is a significant factor for diffusion-controlled and chemically-controlled releases, and this property is investigated in this study. Figure 1 shows a synthetic route for the preparation of dimensionally-stable PILs-based hydrogels consisting of imidazolium-based ionic liquids. Previous work shows the successful embedding of quinine derivatives and enzymes in PILs.[1] Protein kinase inhibitors of type **3** are used as model substances for the swelling and release experiments. PILs have a specific advantage: their porous polymer structure has a very large internal surface. Therefore, their properties, such as the lipophilic/hydrophilic balance, can be tailored by modifying the chemical functionalities. An individualized dosage form for the patient can be achieved through the easy and exact divisibility of mechanically stable hydrogels.

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Figure 1

Figure 1. Synthesis of PILs-based hydrogels; radical polymerization of an imidazolium-based ionic liquid bearing a vinyl group, [VEIm][Br] – 1-vinyl-3-ethylimidazolium bromide, and cross-linker *N,N'*-methylenebisacrylamide (Bis). APS– Ammonium peroxydisulphate, TEMED– *N,N,N',N'*-Tetramethylethane-1,2-diamine, **3** –7-Halogenindol-3-carbonitrile

P62

Thermoplastic polymeric nanofibers - new potential for insulating barriers in biomedical implant applications?

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The improvement of electrical insulation layers for implantable medical devices and electronic implants is demanded for various reasons. Often, implant structures, such as electrical stimulation electrodes, have to withstand mechanical stress due to bending, torsion and tensile loads. Here, implant surfaces or coatings with high durability, extraordinary elasticity and high dielectric strength are required. Furthermore, the permanent mechanical and electrical separation of metal electrodes is another major challenge. In this context, novel composite implant coatings are being developed and characterized based on a combination of insulating and swellable layers as an innovative tool to meet these often conflicting requirements.

In the present study, first investigations of different thermoplastic polymeric nanofibers in combination with hydrogel layers are disclosed. Also, the influence of plasma surface treatment was studied. Different surface properties were detected for thin sprayed or cast polymer film specimens in contrast to nanofibrous structures. The insulation resistance, the dielectric strength, mechanical characterization and the thermal properties as well as the biocompatibility of different plasma activated and untreated thermoplastic polymers and composites were evaluated.

The development of polymer-based composite coatings offers a promising approach by combining advantages of polymers with insulating and swelling properties. In particular, swellable polymers are attractive in terms of self-adjusting spacing of metal surfaces (e.g. electrodes on multi-electrode arrays). Furthermore, the tested polymers show a high potential for improving the fatigue behavior of electronic implant structures in combination with lubricious properties.

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μCT evaluation of 3D printed hybrid materials and hydrogel composites

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Introduction

Synthetic bone substitutes constitute an important group of biomaterials to support the regeneration of bony defects in various clinical applications [1]. Implants for the reconstruction of cranial bone defects should offer different functions which could be realized by a structure of multiple different layers. Therefore, the aim of this project is to fabricate a titanium-hydrogel-hydroxyapatite hybrid structure, which mimics the biological and mechanical functions, necessary for bone healing in this area, by 3D plotting [2]. The evaluation of the implant is performed by μ-Computed Tomography (μCT), which is a novel technique for this biomaterial application.

Materials and Methods

Three layers consisting of a commercially available titanium mesh for the mechanical stability and as building platform, a dense alginate soft layer to prevent soft tissue ingrowth, and a porous alginate-hydroxyapatite composite layer for hard tissue reconstruction were combined by 3D printing technique bioplotting. These materials were scanned with a μCT system in a resolution of 15 μm. The analysis of the data was done with a software developed by Fraunhofer IIS.

Results and Discussion

Due to the μCT measurements three different layers of the 3D bioplotting material can be visualized. The thickness, porosity and position of the layers were measured in the data. With this information it is possible to determine the quality of the 3D printed hybrid materials and the composites of the hydrogel.

Conclusion

The main goal of this study was to evaluate 3D bioplotting hydrogel and composite constructs to achieve superior performance. The μCT data show accurately the structure of the 3D bioplotting material. This technique offers the possibility to evaluate the quality of the 3D bioplotting material.

Acknowledgments

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P64

Agarose-Derived Polysaccharide/Hydroxyapatite Hybrid Materials for Tailored Bone Regeneration

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Polysaccharide derivatives are attractive components for the development of biomaterials used in tissue engineering, particularly bone regeneration due to their inherent advantages such as native biocompatibility and facile preparation by self-assembling compared to synthetic polymer materials. Here, agarose-derived materials are of particular interest due to their ability to generate thermos-reversible hydrogels of non-cytotoxicity. In addition, agaroses offer a broad variety of synthetic methods for functionalization to finally tailor the 3D bulk structure of biomaterials as well as their surface (polarity, topography) [1].

Thus, polysaccharide derivatives are used for scaffold fabrication. Smart scaffolds are able to mimic the natural environment via templated-mediated biomineralization thereby enhancing the osteogenic differentiation of mesenchymal stem cells (MSC) toward osteoblasts. Purinergic receptors (P2x, P2Y) are found to play a key role in osteogenic lineage commitment of human MSCs. Via release of receptor ligands MSC differentiation towards osteoblasts can be triggered [2].

This contribution focuses the synthesis of polysaccharide-derived hybrid scaffolds: polymer analogue derivatization of agarose under homogeneous conditions is studied to generate functionalized agarose derivatives, e.g. tosylated intermediates, azido agaroses and agarose sulfates (analogue to naturally occurring polysaccharide sulfates).

Hydroxyapatite/agarose hybrids are prepared and characterized regarding their chemical composition, 3D bulk and surface (polarity, topography). Methods include FTIR, X-ray diffraction (XRD), X-ray scattering (SAXS), scanning electron microscopy (SEM) and zeta potential; swelling behavior and release of compounds resembling the P2 receptor ligand structure.

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P65

Application of electrospinning for vascular tissue engineering

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Introduction

Coronary artery bypass grafting (CABG) is the most performed open-heart surgery in the world. While the availability of vascular autografts is limited, synthetic grafts suffer from thrombosis and/or hyperplasia. Electrospinning is a promising technique for the generation of novel, anti-thrombogenic scaffolds for vascular prostheses.

Objectives

To investigate the attachment and growth of endothelial cells (HUVECs) and fibroblasts on electrospun poly(ϵ -caprolactone) (PCL) mats with different fiber diameters and novel blend mats containing PCL and silk fibroin (PCL/SF).

Materials and Methods

PCL mats were produced by electrospinning with benign solvents [1], in two different average fiber diameters, namely micro and nano range. Subsequently, the electrospinning method was established and optimized to produce PCL/SF blend fibers. HUVECs and fibroblasts were cultured on the mats for up to 7 days. Morphology (F-actin), viability (calcein) and metabolic activity (WST-8 assay) of cells cultured on the mats of different fiber size and composition were compared.

Results

Metabolic activity assay on day 1 demonstrated that PCL nanofibers and PCL/SF provide a better support for cell growth compared with PCL microfibers. At day 7, the highest metabolic activity in HUVECs and fibroblasts was observed on PCL/SF mats. These results were confirmed by microscopic studies. On day 7, the best support for cell attachment and growth was observed on PCL/SF as compared to PCL nanofibers and PCL microfiber mats.

Conclusion

The mats obtained by blending SF with PCL combine the mechanical benefits of PCL with improved biological functionality of SF. Electrospinning of PCL/SF fibers will be potentially suitable to produce complex 3D vessel-like constructs with small diameter.

Acknowledgments

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Gelatin-Poly(glycerol sebacate) (PGS) Blended Fibrous Scaffolds for Cardiac Tissue Engineering

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Introduction

The very limited regeneration ability of cardiac tissue after an event like myocardial infarction and restricted therapies demand new cardiac tissue engineering (CTE) strategies to repair the damaged tissue. To obtain functional scaffolds, which closely mimic the highly branched myocardial structure and its properties, electrospinning combined with suitable polymers gains increasing interest.

Objectives

This study focuses on the development of electrospun blend fibers of gelatin and PGS prepolymer (PGS_P) or mildly crosslinked PGS (PGS_{MXL}). Gelatin and PGS, also in combination, show beneficial properties for CTE like intrinsic biocompatibility and tunable mechanical properties [1,2].

Materials & Methods

PGS_P and PGS_{MXL} were conventionally synthesized¹ and mixed in different ratios with gelatin using the less toxic solvent formic acid. The electrospinning parameters were optimized to an applied voltage of 15kV, a flow rate of 0.13mL/h, and a distance of 18cm. The mats were crosslinked by glutaraldehyde (GA) vapor and characterized regarding their morphological, physiochemical, and degradation properties in PBS.

Results

Homogenous and defect-free fibers were obtained for all blends with a diameter ranging between 200 and 500nm. The morphology was maintained after GA crosslinking; however the fiber diameter was increased. The presence of both polymers in the blends could be confirmed by FTIR. The mechanical properties were found to be in a range suitable for CTE. Degradation tests revealed a remarkably improved water stability of the blends by GA crosslinking.

Conclusion

After optimization of electrospinning parameters, gelatin/PGS_P and gelatin/PGS_{MXL} blend fiber mats were successfully fabricated. The scaffolds showed interesting properties for future application in CTE.

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P67

Functionalized polymer fibers with extracellular matrix components

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The application of polymer fibers consisting of poly-ε-caprolactone (PCL) and polyglycolide (PGA) has already been widely studied in the field of biomedical sciences; for example as basic material for drug delivery systems or 3D-scaffolds in tissue engineering. They allow an efficient modification of their properties by surface functionalization. Moreover they have shown a good biocompatibility and are biodegradable [1]. The aim of this work is to modify the surface of fibers made from PCL/PGA copolymers in order to test their applicability as components in scaffolds for tissue regeneration. For this purpose, the biodegradable fibers were coated with extracellular matrix components; as one representative there is heparan sulfate. It is well-known in clinical applications as an anticoagulant for wound healing and tissue regeneration [2]. Another promising agent is laminin, which belongs to collagen like glycoprotein and increases the affinity for directing peripheral nerve regeneration [3].

In this work the polymer fibers are functionalized with aminogroups via aminolysis using ethylenediamine. Afterwards the extracellular matrix components are linked to the functionalized fibers [4]. Toluidine blue was used in a colorimetric assay to validate the presence of heparan sulfate on the fibers [5]. Among other things, the attachment and release of laminin was proven by ELISA. Cell culture investigations show promising results.

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P68

Electrospinning of photo-reactive biotinylated chitosan nanofibers – towards selective biomolecule support matrices

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Current applications for enzymes and other biomolecules are limited by losses in activity or difficulties in reusability and handling. Immobilization onto a structural support matrix may help to overcome these obstacles [1,2]. Chitosan nanofibers are promising candidates as immobilization matrices for biomolecules because of their unique combination of molecular-, macro- and nano-properties. The high surface-to-volume-ratio of nanofibers in combination with antimicrobial polycationic nature of chitosan may lead to the development of a new class of biomolecule support [2,3]. However, most of the previously spun chitosan fibers tend to lose structure at aqueous conditions. Covalent modification of chitosan with photo-reactive crosslinking groups [4] before electrospinning helps to solve this problem and provide a UV-light induced crosslinking alternative.

We present a versatile two-step-synthesis route for modification of chitosan from fungal origin with photo-reactive 4-azidobenzoic acid and biotin for selective immobilization of bioactive molecules via biotin/avidin interaction. Subsequent fiber production from photo-reactive chitosan derivatives via electrospinning under controlled environmental conditions yields a fibre mesh which can be photo-crosslinked by UV-light at a wavelength of 265 nm. These hydrophilic but water stable nanofibers show biotin groups at their surface which is quantifiable via HABA-Avidin-Assays. Such groups can subsequently be used for selective immobilization of various biotinylated biomolecules via avidin as a mediator.

Figure 1: Production process of biotinylated chitosan nanofibers as biomolecule support matrix.

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Figure 1

P69

Shear rheology of nanofiber reinforced alginate solutions for bioprinting

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In recent years, bioprinting has become an important approach for creating customized tissue replacements used either for healing or replacing damaged human tissue and cartilage [1,2]. One of the crucial points in bioprinting is cell proliferation, which is highly influenced by the shear stresses occurring during the printing process [3]. Not only the cell proliferation, but also the rheological behavior of hydrogel solutions depends on shear stresses occurring in nozzle geometries. Both extrema, too high and too low viscosity would impede a successful printing result. In theory both restrictions can be bypassed by fiber reinforcement. The stiff fiber segments can be utilized to mitigate shear stresses on cells and also to adjust rheological properties. In this work the influence of PCL nanofibers, fabricated by e-spinning, on the rheological behavior of alginate solutions is investigated. Therefore different solution concentrations (ranging between 10-50 g/l) with distinct fiber concentrations are characterized using plate-plate-rheology. The results will provide promising insights on the rheological properties of fiber filled bioinks.

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P70

Investigation of nanoferrite-based inductive fibres for applications in hyperthermic ablation therapy

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Cancer is the second leading cause of death globally, and was responsible for 8.8 million deaths in 2015 (World Health Organization). The tumour often infiltrates and narrows vessels and hollow organs and is usually removed surgically. Local recurrences often lead to reocclusion or restenosis. Currently, conventional metal stents or plastic tubes are used to keep the affected hollow organs open. These have the disadvantage of lacking the ability to keep the hollow structures open in the long term. Using local hyperthermia, mediated by inductively heatable nanoferrite fibres, the tumour could be locally destroyed several times if necessary and thus the hollow structure can be reopened in the sense of "self-cleaning". The changing magnetic field is applied externally so that the treatment is non-invasive. For this purpose, the production process of nanomodified fibre-based implants for use in hyperthermic ablation therapy and permanent retention in the body is being investigated in the present study. These fibres are to be produced and further processed into an implant for use in ablation therapy and permanent retention in the body. The technical challenges in the development of nanoferrite fibres lie in the homogeneous particle distribution without macroscopic agglomerates in the nanocompound and in the continuous production of nanoferrite fibres in the melt spinning process. The intended research result is the development of a platform technology for the treatment of restenosis due to tumour growth.

First of all, nanocompounds consisting of a thermoplastic polymer and different concentrations of nanoparticles are produced at various compound feeding rates and temperatures. The effect of particle concentration, mass flow and melt temperature is evaluated with respect to particle distribution and inductive heating. The nanoparticles used in this study are synthesized at the Institute of Applied Medical Engineering of RWTH Aachen University.

P71

Nanopatterned Ti surfaces for investigating the effect of surface topography on protein adsorption

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Titanium, usually with a passivating layer of TiO₂, is a widely employed implant material, due to its superior mechanical properties and biocompatibility. To improve the implant-tissue interaction, previous studies have focused on enhancing the metal surface's bioactivity by chemical and mechanical treatments.] Recently, however, also the influence of topographical features at the nanometer scale on protein adsorption and cell response has been recognized [1].

To study the influence of nanoscale topography on the specific and nonspecific adsorption of selected proteins, regular nanoscale ripple patterns on Si wafers have been fabricated. Such ripple patterns form spontaneously by self-organization on the Si surface during off-normal irradiation with low-energy Ar⁺ ions. The periodicity of the ripple patterns depends on the ion energy and can be tuned between few ten and several nanometers [2].

Of particular focus for this work is the possibility to use the rippled surfaces as substrates for the growth of thin films. In particular, thin titanium films with 20 nm thickness are deposited by magnetron sputtering which under optimized conditions may perfectly reproduce the ripple patterns [3]. With this approach, various topographically nanopatterned surfaces with identical surface chemistry can be fabricated. These nanopatterned Ti surfaces with various pattern periodicities between 20 and 50 nm are then utilized for investigating the effect of nanoscale surface topography on protein adsorption by ellipsometry.

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Figure 1

P72

Modification of Neural Electrode Surfaces by Nanoporous Platinum Coatings

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For the improvement of the function of neural electrodes, the long-term biointegration as well as the interface between electrode and nerve fibers needs to be improved. This can be realized by chemical modification of the electrode surface or by integrating a drug delivery system e.g. in nanoporous platinum (NPt) coatings on electrode contacts. For loading pores with active agents of various size, the pore diameter is adjusted by use of templates like Pluronic®F127 and polystyrene latex beads (PLBs). Potential active agents for the application in the inner ear are rolipram and BDNF, which induced enhanced spiral ganglion neuronal survival [1].

NPt is deposited electrochemically on dense platinum. Within the first method, Pluronic®F127 is dissolved in Pt(IV)solution. During the platinum deposition, Pluronic®F127 is incorporated into the forming platinum coating and removed subsequently. Within the second method, dense platinum is coated with PLBs. After template formation, platinum is deposited in the voids of the PLB layer and PLBs are removed subsequently. The NPt coatings were characterized by SEM, krypton sorption measurements, impedance spectroscopy, and cell culture investigations with NIH3T3 fibroblasts, SGNs and BDMSCs.

Both methods lead to NPt. By using Pluronic®F127 small pores with a size of about 10 nm are obtained, by using PLBs the large pore diameter corresponds to the bead size of 50 nm. Impedance measurements of the NPt coatings show improved impedance behavior in the lower frequency range for nanoporous platinum with smaller as well as larger nanopores. Cell culture investigations indicate a good cell compatibility and SGN neurite growth. Rolipram was successfully released from the NPt exhibits smaller nanopores.

This work was supported by the DFG Cluster of Excellence EXC 1077/1 "Hearing4all".

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Figure 1

P73

Ion release and protein adsorption capacity of additive-free metal nanoparticles-alginate composite materials with high particle mass load

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Nanocomposites are highly interesting for biomedicine as the embedded nanoparticles can add novel functionality to the polymer. In this context, alginate is ideally suited as a matrix biomaterial, due to its abundance, low price, simple and non-cytotoxic gelation process and its widespread application e.g. in tissue engineering. In previous experiments, we demonstrated laser ablation and in situ embedding of nanoparticles into the alginate is a versatile strategy for nanocomposite synthesis. In these studies we found that iron nanoparticles, embedded into the alginate hydrogels, had benign effects on adhesion of endothelial cells, probably due to Fe ion release. However, iron mass loading was relatively low in these studies [1]. In this work, the loading of gold and iron nanoparticles into alginate by a laser-based in situ embedding method is systematically studied. We demonstrate how the loading of the particles into the alginate can be controlled and optimized, utilizing high power lasers with high productivity. [2] In consecutive experiments we realize the parallel embedding of two different nanoparticles, gold and iron, into the alginate. This could be particularly interesting as both elements were shown to enhance the cytocompatibility of biomaterials by two different modes of action, Fe probably by iron ion release [1] and Au by altering the surface charge of polymers. [3] In this study we examine the release of bioactive iron ions in correlation with the particle mass load. Finally, we evaluate the adsorption of model proteins found in the ECM (collagen or fibronectin) as an initial indicator for potential cytocompatibility and evaluated to what extent affinity to these proteins is correlated with the loading and composition of the composite biomaterial.

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P76

3D printed composite material for bone replacement - quantitative evaluation of osseointegration in an animal model

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Background: For patient specific replacement of calvarial and facial bone, a novel composite implant material consisting of 3D printed calcium phosphate (beta-TCP) and a methacrylate copolymer was developed. The additive technology enabled efficient manufacturing of complex shaped patient specific implants (PSI). The material showed suitable properties in mechanical tests and in vitro assays. **Methods:** To evaluate in vivo biocompatibility, experiments were conducted in 30 NZW rabbits according to the “Jenaer Schädelmodell” [1]. In rabbit calvaria, implants were applied in trepanation holes: the composite, its polymer component, TiAl6V4 and the Bioceramic Bioverit®. After 9 and 18 months, respectively, calvaria with implants were explanted. μ CT scans and hard tissue histology were performed [2]. Both imaging modalities were used to evaluate osseointegration. 2D morphometry software HistoGap was developed and used on the histology images to measure bone-implant distance in detail. 3D μ CT images were processed in Avizo (ThermoFisher). To obtain partial bone volume (BV/TV) as function of distance from implant, an algorithm was defined in Avizo and applied on segmented μ CT scans. **Results and discussion:** The combined evaluation of 2D histology and 3D μ CT images enabled comprehensive qualitative and quantitative assessment of osseointegration. All tested implant materials performed well in the in vivo assay. Quantitative evaluation revealed a significantly better osseointegration of the new composite material in comparison to TiAl6V4 and Bioverit® which have proven successful in many years of clinical practice. Good osseointegration is a significant indicator for weak foreign body response leading to good tissue integration of the material, thus minimizing the risk of biofilm formation [3].

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Figure 1

fig.1: Microphotograph of composite implant, osseointegrated in rabbit calvaria
(hard tissue histology, mod.Masson-Goldner staining; bone: dark grey)

Figure 2

fig.2: (a) segmented μ CT scan of composite implant, surrounded by concentrically arranged volumes of interest (VOI); (b) volume rendering of rabbit calvaria bone in one of these VOI; (c) bone volume fraction (BV/TV) of the VOI, unrolled to the plane

P78

The chondrogenic potential of stromal vascular fraction cells (SVF) for cartilage reconstruction using 3D bioprinting strategies

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The reconstruction of cartilage defects is still a challenge in reconstructive surgery. Traditional strategies show significant limitations including restricted availability of donor tissue, donor site morbidity, formation of inadequate replacement matrix, deficient aesthetic outcome and loss of implanted cells. Tissue engineering methods combined with 3D printing provide a suitable tool for the production of patient-specific cartilage implants. It allows a layered and zoned, accurate deposition of cells and biomaterials, essential for tissue reconstruction.

The aim of the project was to investigate, *in vitro*, the potential of SVF and adipose derived stem cells (ASC) in co-culture with human nasoseptal chondrocytes (hNC) for cartilage-specific matrix formation in 3D bioprinted constructs. SVF were isolated from adipose tissue derived from liposuction. Cells (hNC, control) and co-cultures (hNC/SVF, hNC/ASC) were mixed with nano-fibrillated cellulose and alginate (NFC-A). Grid-shaped constructs were printed with an initial cell number of 10×10^6 cells and cultured for up to 28 days in chondrogenic medium. Cell viability was assessed by live/dead staining, proliferation examined by CyQuant-Assay. *Neo*-synthesis of cartilage-specific components such as collagen type II (Col II), SOX-9 and proteoglycans (PG) was examined by immunohistochemical staining and quantitative DMMB-Assay.

All constructs revealed a high cell viability during culture for up to 28 days. In SVF/hNC as well as in hNC/ASC co-culture constructs, PG-, Col II- as well as SOX-9-production was detected. Likewise, constructs with hNC alone exhibited a *neo*-synthesis of cartilage-specific extracellular matrix. A higher chondrogenic capacity was found for hNC/SVF co-cultures compared to hNC monocultures or hNC/ASC constructs.

The results show that hNC/SVF co-cultures offer chondrogenic potential in NFC-A hydrogels and represent a suitable cartilage regeneration system combined with 3D printing.

P79

Combined 3D-Printing and Cell Sheet Engineering Approach Towards an *in vitro* Kidney Model

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Question

The renal proximal tubule (PT) is a major target of nephrotoxicity due to its role in blood clearance of e.g. xenobiotics. Therefore, there is a high demand for human *in vitro* models that reflect the PT physiology and accurately predict the effects of xenobiotics on the PT. Our aim is the development of an advanced model of the PT to overcome drawbacks of current static 2D-cultures and animal models by combining cell sheet engineering and 3D-scaffold printing.

Methods

We established functional thermoresponsive polyglycidyl ether (PGE) coatings [1,2,3] for the detachment of human renal proximal tubule epithelial cells (RPTEC) and HUVECs as mono- and bilayers. The cell sheets were characterized via immunofluorescence, TEER-measurement and qPCR and the impact of dynamic culture on cell orientation and cell sheet detachment was evaluated (Fig.1).

Results

The PGE coatings allowed for the successful detachment of epithelial and endothelial cell sheets. The functionality of the cell sheets regarding polarization, expression of transporters (OATs, OCTs) and barrier integrity was demonstrated.

Conclusion

With our thermoresponsive PGE coatings, we were able to harvest various functional cell sheets. The combination of these cell sheets with 3D-printed scaffolds will allow the development of an advanced 3D-model of the PT for further dynamic maturation and future nephrotoxin detection.

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Fig.1. HUVECs cultivated under dynamic perfusion conditions for 13 h at 5 dyn/cm² (white arrow represents direction of flow).

Figure 1

P80

Labbag® - A closed and surface based system to cultivate human stem cells in hanging droplets

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Human induced pluripotent stem cells (hiPSCs) have great potential to reduce the number of animal tests in drug discovery or to create meaningful data to understand diseases, especially when three dimensional cell models consisting of differentiated cells are regarded. The processing steps for generating and storing these cells include cell expansion, embryonic body formation (cell aggregates), differentiation and cryopreservation. The formation of the 3D aggregates is the essential step to start cell differentiation or micro tissue generation. A standard method for aggregate formation is the so-called cultivation in "hanging droplets". Current techniques based on manual pipetting are time-consuming or very expensive when pipet robots are used.

An innovative new approach is the Labbag® - a closed plastic bag cell cultivation system developed by an interdisciplinary team of three Fraunhofer institutes. In this bag hundreds of droplets for 3D aggregate formation can be created within seconds. This is possible, because the bag provides a patterned surface with different wettability. Surface properties are tailored by coating the surface of the bag using an atmospheric pressure plasma process. In this two-step process at first a cell culture medium repellent superhydrophobic surface was created, following by a patterned coating of hydrophilic spots. The cell suspension has to be swirled over the bag surface, then the bag is turned around and hundreds of droplets have been created. Within these hanging droplets hiPSCs are able to form 3D aggregates. Due to the materials chosen, the Labbag® does not only serve as a 2D and 3D cell cultivation system, but also cryopreservation is possible within the bag. Thus, this system can be used in the whole workflow for processing stem cells. One of the biggest advantages is that the bag is a closed system and by this means we create a GMP conform environment.

P82

Poly-Phosphate-modified PCL-scaffolds modulate microvascular integration *in vivo*

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Poly-phosphate (polyP) is involved in various biological processes such as inflammation, complement activation and tissue regeneration and it could be shown that it plays a crucial role in the context of bone mineralization/regeneration, metabolism and angiogenesis. Since bone regeneration and angiogenesis are promising approaches in regenerative medicine, the aim of this work was to investigate the effect of polyP-modified polycaprolactone scaffolds on the angiogenesis *in vivo* using the chorioallantoic membrane Assay (CAM).

PolyP-modified PCL-scaffolds were 3D printed using a 3D-bioplotter (EnvisionTEC, Gladbeck; Germany) and the bioplotter RP 3.0 software as described earlier. For the *in vivo* experiments the printed scaffolds were placed on the CAM and investigated after 24 h, 3 days and 7 days of CAM incubation, unmodified PCL samples were used as control. For analyses of angiogenesis, samples were investigated light microscopically, fluorescence microscopically using FITC-dextrane and immunohistologically using anti- α -SMA staining.

The results demonstrate that within the first 3 days of scaffold incubation no distinct effect can be observed in the polyP-modified samples compared to control. However, after 7 days the comparison of the modified samples and the control samples reveal a decreased number of vessels on the analyzed micrographs after incubation with the polyP scaffolds. The immunohistological analyses and the intravital microscopy confirm these findings with an increased number of branches in the control compared to the polyP samples. According to literature the reduction of the vessel number and the branches may be correlated to the pruning of vessel sprouting and the formation of functional vessels. In order to understand the mechanisms of the polyP effect in the context of angiogenesis further experiments will be necessary.

P83

Identification of relevant angiogenic factors in the context of tissue engineered pre-vascularized mucosa transplants based on collagen

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The reconstruction of mucosal tissue defects is an issue of great interest in reconstructive surgery. A promising approach for the wound coverage after surgery is the use of artificially generated pre-vascularized tissue equivalents. We developed a pre-vascularized tissue equivalent in a tri-culture of primary buccal epithelial cells, fibroblasts and microvascular endothelial cells successfully, based on the collagen matrix Bio-Gide® from Geistlich. However, the generation of pre-vascularized structures within the tissue equivalent was restricted to only superficial areas of the matrix. It is not completely understood yet, why the used endothelial cells did not migrate in depth of the tissue equivalent in order to form vascular structures. To understand the cell biological background for the reduced migration of endothelial cells, we investigated the intercellular communication in co-cultures of primary microvascular endothelial cells and buccal fibroblasts incubated on the used collagen matrix. The secretion patterns of the cells were investigated using a multi-cytokine ELISA in which 32 angiogenic factors were analyzed after 3 days and 21 days. The results show that compared to day 3, on day 21 the secretion levels of 26 angiogenic factors were increased: IL-8 and IL-2 showed the highest secretion levels followed by Rantes, Leptin, IL-17a and VEGF on day 21. The complex intercellular communication could be demonstrated impressively. The significantly increased secretion levels of factors such as the Interleukins 8, 2 or 17a show that these factors seem to play a crucial role in the context of intercellular communication between endothelial cells and fibroblasts. However, the mechanisms of cellular interactions with respect to pre-vascularization in complex co-cultures is not completely understood in detail and further investigations will be necessary to analyze the impact of single cytokines.

P84

Development of Artificial Vascularized Scaffold for Tissue Engineering

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Introduction

Tissue engineering involves the fabrication of 3D scaffolds and the implementation of desired cells in the scaffold. Non-vascularized tissue constructs are restricted to dimensions of a few hundred micrometres due to the diffusion limit, which also limits their clinical use. Though different strategies were developed to generate vascularized scaffolds, these are not yet successful in including a closed perfusion system.

Objectives

The aim of this project is to develop a mature and perfusable human endothelial cell network within a 3D construct applicable for clinical use. For that, the initial points are addressed: a) hydrogel generation from xeno-free components, b) endothelial cell network generation of relevant size for its subsequent perfusion.

Materials and Methods

Human endothelial cell network is generated by a self-assembly of human umbilical vein endothelial cells (HUVEC) in co-culture with human adipose-tissue derived stromal cells (hASC) in a human collagen I gel. The hydrogel/cell mix is casted onto decellularized small intestine submucosa (SIS) and cultured in EGM-2. The construct thickness was investigated upon addition of SeaPlaque™ agarose up to 0.4% to the hydrogel.

Results

The generation of a stable endothelial cell network is observed using human fibroblasts collagen I as the main matrix component and xeno-free alternative to the previously used matrigel. The addition of 0.4% SeaPlaque™ agarose to the cell/hydrogel resulted in an increase of approximately 50% of the construct thickness.

Conclusion

The human collagen I as a main matrix protein seems to be sufficient for the support of an endothelial cell network formation. Moreover, it can be concluded that the thickness of the construct can be manipulated by addition of SeaPlaque™ agarose.

P85

Characteristics of osteoblasts on titanium surface modified by nanotubes

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Introduction

Titanium and its alloys are used in orthopaedic implants due to their biocompatibility, low toxicity and excellent mechanical properties. Titanium nanotubes provide a surface modification with unique chemical and physical characteristics allowing an increased interaction between the surfaces of the implant and surrounding cells and cell proliferation. Final structure and dimensions of nanotubes characterise the surface properties, and can be altered during the process of anodic oxidation.

Objectives

Our objectives are to compare the growth of the cells on unmodified and modified titanium surfaces with a stress to the cell behaviour.

Materials and Methods

The nanotubes on the titanium surface were created by the anodic oxidation. Material surface was characterized by Scanning Electron Microscopy and Atomic Force Microscopy. Two human osteoblasts cell lines were cultivated (physiological HOb vs. osteoarthritic HObA) under standard conditions.

Results

Nanotubes (NT) were produced in a diameter from 20 to 150 nm and the height from 102 to 160 nm, depending on the voltage and on time. The cell size increases with the modification, and even with the size of the NTs till the NT diameter 100 nm. For the diameters over 100 nm, the cell size decreased. Furthermore, according to the AFM, the stiffness of the cells differ between unaffected and osteoarthritic cells. For further analyses on cells the NT with a diameter 70-100 nm were selected. The increased proliferation of the physiological cells was detected on the modified surface.

Conclusions

We described the differences in the cell growth and cell characteristics based on the modifications of material surface. The results could contribute to finding a new surface modification or improvement in terms of the advanced mechanical and biocompatible properties.

P86

Controlled Cell Activity on Poly Electrolyte Multilayer Functionalized Gold Surfaces

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Surface-tissue interactions can be directly controlled by surface modifications where the contact interface is precisely tuned according to desired purpose. Micro- and nanoscaled surface properties affect cellular adhesion and proliferation behavior.

We are focusing on understanding of biological response to specific surface properties from the cellular perspective. The goal is the development of a surface coating which can control cellular behavior and can be applied in implantology.

Polyelectrolyte multilayer coatings (PEM) prepared via layer-by-layer deposition technique enable formation of well-defined surfaces with high variability of surface properties. In our work we demonstrate a correlation between physico- chemical properties of PEM and adhesion and proliferation of cells on these surfaces.

Coatings based on the same couple of polyanions and polycations were prepared. By changes in salt concentration of the deposition solutions molecular interactions between polymer chains are shielded. This results in variation of the film thickness and its viscoelastic properties. The amount of deposited mass, film thickness and viscoelastic properties of the PEM coatings on gold substrates were characterized utilizing Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D). The response of the cells to the coatings was monitored by fluorescence microscopy and Resazurin assay.

Cellular activity is increased on PEM coated gold substrates in comparison to uncoated substrate. The highest activity shows on cationic low salt coating and on anionic high salt coating. This effect does not correlate with the difference in medium protein adsorption. Other factors should be responsible for the change in the cell adhesion. Results show that viscosity and shear storage modulus of PEM coatings increase in accordance with salt concentration of coatings solutions indicating that the viscosity and shear storage modulus might affect the cellular activity on surfaces.

P87

Biofunctional coating with anti-thrombogenic activity

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Objective

Biomaterials in contact with blood bear the risk to produce a thrombus, or clot. Thrombi could become detached and travel through the bloodstream, causing cerebral infarction. Our approach is to prevent the danger of formation of thrombi by establishing a biofunctional coating, applicable on cardiovascular implant materials, such as electrospun polycarbonateurethane (PCU).

Methods and Results

Thin coatings were produced, using the layer-by-layer technology, that is sequential deposition of biocompatible, degradable polymers that physically bond together and heparin as an active component. The coatings were characterized concerning polymer deposition using fluorescently labelled polymers, heparin incorporation by quartz crystal microbalance, and changes in surface energy by contact angle measurements. All analyses showed a constant and reproducible build-up and efficient immobilization of heparin.

Analyses with human plasma showed a clear retardation of the coagulation process by the before established coating, quantified by turbidity measurements. This desired effect was well preserved even after sterilization of the coating with ethylenoxide and could be further enhanced by increasing the coating thickness, confirmed by ellipsometry measurements.

Using fluorescently labelled polymers, the successful transfer on the electrospun PCU membrane could be shown, a material applicable for cardiovascular interventions. The biofunctionality of the coating, when deposited on this material, could be confirmed as well by additional coagulation measurements.

Conclusion

A new biofunctional coating could be established and characterized successfully, showing favorable anti-thrombogenic properties. In combination with biomaterials, such as the tested electrospun membrane, it can be used, e.g. as covering layer for cardiovascular devices.

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P88

Biomaterial induced multinucleated giant cells express proinflammatory signaling molecules: A histological study in human

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Introduction

After biomaterial implantation, the initial cellular reaction to the biomaterial depends on its physicochemical properties. In addition to macrophages, multinucleated giant cells (MNGCs) were frequently observed in response to different bone substitute materials. The present study evaluated the inflammatory pattern of macrophages and MNGCs in response to two different Bone substitute materials in human biopsies.

Materials and methods

Bone biopsies were gained during a clinical study using a split mouth design to evaluate the regeneration capacity of two bone substitute material (synthetic bone substitute material (SBMS) = NanoBone® vs. xenogeneic bone substitute material (XBSM) = Bio-Oss®) in comparison to the non-augmented region. Immunohistochemical markers were used to evaluate the pro- (M1) and anti-inflammatory (M2) polarization (CD-68= macrophages; CCR-7 and Cox-2 (M1) and CD-206 and CD-163 (M2) and tartrate-resistant acid phosphatase (TRAP)). The results were quantified histomorphometrically and analyzed statistically.

Results

The macrophage polarization pattern in SBMS showed a significantly higher number of M1 cells than did XBSM and non-augmented bone. XBSM induced a significantly higher number of CD-206-positive macrophages than SBMS did. No significant difference was found between XBSM and the non-augmented bone.

MNGCs expressed CD-68 and TRAP. In both test-groups, MNGCs showed a high proinflammatory character (CCR-7 and Cox-2-positive) and their number in the SBMS group was significantly higher than that of XBSM. The tissue distribution showed a significantly low percentage of the remaining biomaterial in SBMS compared to XBSM.

Conclusion

Within the limitations of this study, these findings show that MNGCs exhibit a rather proinflammatory character and lead to biomaterial degradation, once they are induced in a high number. The premature degradation of BSM is compensated with connective tissue and not new bone formation.

P89

***In vitro* und *in vivo* biocompatibility analysis of a novel bone block consisting of porcine collagen and a synthetic biphasic bone substitute as an alternative concept to allogeneic materials**

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Introduction

In the present study the cell- and tissue reactions to a new bone substitute block made of synthetic biphasic hydroxyapatite/ β -tricalcium phosphate (HA/ β -TCP) granules (BCP) embedded in a naturally crosslinked porcine collagen matrix (maxresorb® flexbone) were analyzed *in vitro* and *in vivo*. A collagen containing cancellous allogeneic bone block (maxgraft®) and a synthetic biphasic bone block (maxresorb®) were used as controls. It was hypothesized that maxresorb® flexbone should exhibit regenerative properties comparable to allografts.

Methods

An osteoblast cell line, MG-63, was used to evaluate the adherence and proliferation of the cells on the biomaterials. Cells were added and at specific time points after addition were examined morphologically and stained to determine distribution and expansion on the materials. Proliferation and cytotoxicity assays were used to and compare the growth rate of cells and cell compatibility. For the *in vivo* study the three materials were subcutaneously implanted in 45 rats for up to 60 days. Specialized histological and histomorphometrical methods were applied for analysis of the tissue response.

Results

The *in vitro* results showed that the novel bone block led to a comparable adherence, cell morphology and proliferation of osteoblasts as in contact with the allogeneic material and only slight differences were observed in the proliferation of cells. The *in vivo* results showed that the inflammatory tissue reactions, i.e., the numbers of MNGCs were comparable and significantly decreased compared to the numbers on the BCP group. Moreover, the implant bed vascularization was superior to the BCP material alone and comparable to the allogeneic bone.

Conclusion

The results show that the novel bone block composed of BCP granules and native porcine collagen appears to be a favorable alternative to non-load bearing allogeneic bone grafts as both the regenerative potential and the integration behavior were similar.

P90

Histological analysis of bone regeneration with different doses of rhBMP-2 in an ovine lumbar interbody fusion model

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The objective of this study was to investigate the effects of different doses rhBMP-2 on bone healing in an ovine lumbar interbody fusion model.

Methods

22 sheep underwent two level lumbar interbody fusion using a ventrolateral approach with secondary dorsal fixation. PEEK-cages filled with rhBMP-2 (0,5mg; 1mg; 2mg) or empty PEEK-cage or ACS filled cage were implanted. Histology was performed after 3 and 6 months.

Results

Good fusion results were detected in the rhBMP2 groups after 3 and 6 months.

The highest BMP 2 group (2mg) showed higher inflammatory reaction in the cage after 3 months than less BMP2. A compromised bony structure were observed in 7/8 BMP2 treated segments around the cage.

At 6 months most of the segments with an empty cage or cage/ACS showed a chronic inflammation. Predominant cells were macrophages and giant cells. The groups treated with rhBMP-2 showed only a few mild chronic inflammatory reactions.

Discussion

The dose dependent effect of rhBMP-2 on bone healing could also be recognized in our study. Attention has to be paid for the proinflammatory properties of the growth factor. Also the potential for causing transient bone resorptions was demonstrated. The compromised peri-implant bone in the segments treated with rhBMP-2 can be concluded that there were bone resorptions which already merged into an increased osteoblastic activity. This finding wasn't recognized at 6 months anymore.

Striking is that at 6 months most of the segments without rhBMP-2 showed a compromised bone structure in and around the implant with a mainly moderate chronic inflammatory reaction. Therefore, the cage as a reason must be considered and it has to be questioned whether PEEK is the optimal material for interbody cages.

P91

Development of a volume stable barrier membrane for Guided Bone Regeneration based on a HF-coated magnesium mesh combined with a collagen membrane: *In vivo* biocompatibility, tissue response and bone regeneration

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Introduction

Barrier membranes are standard biomaterials for guided bone/tissue regeneration. Resorbable collagen membranes exhibit poor structural properties, while volume stable membranes are non-resorbable and require surgical removal. Magnesium is a favorable biomaterial for the development of stabilizing structures. However, it's premature degradation behavior requires a strategy to delay hydrogen release. The aim of this work was the *in vivo*-investigation of a volume-stable barrier that combines a collagen membrane with a magnesium mesh coated by hydrofluoric acid (HF).

Methods

In 18 rabbits, four calvarial defects were randomly covered by magnesium reinforced collagen membranes uncoated or coated by HF or a pure collagen membrane. One defect served as empty control. After 6, 12 and 18 weeks, the explants of six animals were examined by DVT, contact radiography and histology for bone regeneration, degradation behavior of the membranes, development of hydrogen gas cavities and inflammatory reactions.

Results

The two variants of the magnesium-reinforced membranes were detectable until the end of the observation period, while the collagen membrane was not observable. No significant differences ($p > 0.05$) regarding bone regeneration were found. In defects covered by the magnesium-reinforced membranes, residuals of the meshes were observed. The coating was degraded by mononuclear cells up to 12 weeks, while the uncoated magnesium meshes elicited a fibrosis-like tissue reaction. Starting from 18 weeks, the tissue reaction in both groups were similar. Both magnesium meshes were associated with gas bubbles, while they decreased over time and did not interfere with bone regeneration.

Conclusion

The results show that the magnesium-reinforced membranes show comparable regenerative properties as simple collagen membranes. Combined with volume-stable properties, this new developed membrane can be classified as an optimal equivalent for GBR/GTR therapy.

P92

Effect of the composite monomer triethylene glycol dimethacrylate (TEGDMA) on angiogenic differentiation of dental pulp stem cells (DPSCs)

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Objectives

The co-monomer most commonly used in dental composites is triethylene glycol dimethacrylate (TEGDMA). Numerous studies have shown cytotoxic effects of TEGDMA. In this study we investigated the influence of TEGDMA on the angiogenic differentiation potential of dental pulp stem cells (DPSCs).

Methods

DPSCs were isolated from third molars. For angiogenic differentiation, DPSCs were cultured in angiogenic differentiation medium (ADM) or in control medium (CCM) for up to 28 days. In addition, the cells were treated with 0.1 mM or 0.25 mM TEGDMA. RNA was isolated from DPSCs prior to angiogenic differentiation (d0) and at days 7, 14, 21 and 28 after angiogenic induction. Subsequently, qPCR analyses were carried out measuring the expression of angiogenic marker genes (PECAM1, KDR, FLT1, VEGFA). Furthermore, we carried out a spheroid sprouting assay on Matrigel with pre-cultured DPSCs grown for 14 days in ADM or CCM with or without TEGDMA.

Results

The expression of PECAM1 (day 28, 16 fold), KDR (day 28, 19 fold) and VEGFA (day 28, 4 fold) increased significantly over time in DPSCs grown in ADM as compared to DPSCs grown in CCM. TEGDMA at 0.25 mM showed a strong inhibiting effect on the expression of PECAM1 and VEGFA for cells grown in ADM and on the expression of FLT1 for both cells grown in ADM and in CCM. A marked Spheroid Sprouting is seen for DPSCs pre-cultured in ADM but not for DPSCs pre-cultured in CCM.

Conclusions

Our in vitro experiments suggest the ability of DPSCs to differentiate toward the angiogenic lineage. TEGDMA (0.25 mM) attenuated angiogenic marker gene expression pointing to an inhibiting effect of TEGDMA on the angiogenic differentiation process of DPSCs. Angiogenesis is a prerequisite of successful tissue engineering. Therefore a comprehensive understanding of the interplay of TEGDMA and DPSCs is of high importance for future clinical applications.

P93

Impact of copper on osteogenic induction strongly depends on osteogenic supplements and differentiation status of human mesenchymal stromal cells

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INTRODUCTION

Copper ions are supposed to improve osseointegration in vivo when incorporated into implant coatings. However, data on *in vitro* characterization differ much with respect to cell growth and osteogenic potential which might be based on differences in cell types and osteogenic supplements. This work is dedicated to identify the role of the differentiation status of human mesenchymal stromal cells as well as different osteogenic supplements in presence of copper ions.

EXPERIMENTAL METHODS

Copper ions (0 - 50 μ M) were added either directly after cell seeding or after 11 days of pre-differentiation. Osteogenic induction varied in ascorbic acid (ASC) concentration and formulation (0 - 300 μ M ASC or 50 μ M ASC phosphate).

RESULTS AND DISCUSSION

While addition of copper to basal medium had no adverse impact on cell growth, in presence of standard osteogenic induction with 300 μ M ASC growth was inhibited and only ~70% of initially seeded cells survived (Fig. 1A). If copper was added to pre-differentiated cells they further proliferated but slower than control. In presence of copper, ALP level remained comparable to the starting level with or without osteogenic supplements (Fig. 1B), while for pre-differentiation a slight decrease compared to the much higher level at the time point of copper addition occurred. Mineralization was decreased in presence of copper when added from day 0 but in case of pre-differentiation copper addition even topped the control (Fig. 1C).

Stable ASC phosphate in lower concentration compared to ASC provoked similar osteogenic potential and reduced the adverse effects, but a concentration dependent impact of copper was still visible. Reactive oxygen species were identified as potential reason for impaired cell behavior and their concentration varied depending on actual ASC and copper concentration.

ACKNOWLEDGMENTS

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Figure 1

Fig. 1 Proliferation and differentiation behavior of hMSC in presence of copper ions cultured either in basal medium or with differentiation medium (300 μ M ASC) or postponed administration of copper ions to pre-differentiated cells

P94

Storage stability of biomaterial eluates and the influence on cell viability

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Implant materials for medical use need to be easy to use and autoclavable without changes in structure and stability. In particular the biocompatibility has to be tested in vitro according to DIN-EN ISO 10993, in a direct contact assay or with corresponding eluates of the biomaterial. Until now, there is no standard specification for storage and a later use of eluates. Thus, aim of this project was to evaluate storage stability of biomaterial eluates in dependence on storage time and storage temperature. To test this, we used materials with known biocompatibility in cell culture. Epoxid-based polymer material reveals high cytotoxicity in vitro, whereas electrospun polylactide non-woven as well as tricalcium phosphate (solid and powder) shows low cytotoxicity and thus can be mentioned as biocompatible. Storage stability and thus biocompatibility of the stored eluates was evaluated by viability assay (WST-1) and cytotoxicity assay (LDH) after a storage time of 1 and 7 days at different temperatures (4°C, -20°C), in comparison to freshly prepared eluates. Microscopic valuation and cytotoxicity analyses approved the expected biocompatibility of the evaluated materials. Analyses of the storage stability revealed higher cell viability after an eluate storage at -20°C. A longer storage at 4°C led to an increase of cytotoxicity, except for the epoxid-based polymer material. Possible reasons could be chemical reactions and degradation processes of the dissolved components of the eluate as well as changes in the pH value. Consequently, long time storage of eluates at higher temperature leads to lower biocompatibility. Thus, preliminary studies for storage stability of eluates are mandatory.

P95

HEMA induces oxidative stress and modulates the transcription of genes related to inflammation and the organization of the extracellular matrix in human gingival fibroblasts and oral keratinocytes

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Question

2-hydroxyethyl methacrylate (HEMA) is a co-monomer of resin composite materials widely used in dental restorative therapy. Unpolymerized HEMA elutes from dental materials into the oral environment. Aim of our study was to analyze the impact of HEMA on the transcription of genes from cellular processes that are involved in the maintenance of redox and tissue homeostasis: response to oxidative stress, regulation of inflammation and organization of the extracellular matrix (ECM). Human gingival fibroblasts (HGFs) and oral keratinocytes (OKF6/TERT2) as first target cells of eluted co-monomers were used for the analyses.

Methods

HGFs and OKF6/TERT2 cells were grown in monolayer cultures and treated for 24 h with different concentrations of HEMA (0.5 - 10 mM). Hoechst 33342 and LDH assays were used to determine HEMA-caused cytotoxicity. Quantitative RT-PCR was used to assess alterations in gene expression of genes related to oxidative stress, inflammation and the organization of the ECM.

Results

HEMA caused cytotoxicity in HGFs and OKF6/TERT2 cells in a concentration dependent manner. Gene expression analyses showed that genes from the analyzed processes were regulated in both cell types when treated with HEMA. For instance, genes related to defense against oxidative stress, like heme oxygenase 1 (HO-1) and NAD(P)H quinone dehydrogenase 1 (NQO1), were strongly induced. mRNA abundances of the pro-inflammatory cytokine interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α) were reduced. Genes related to the organization of the ECM, like collagen I (COL1A1), matrix metalloproteinase 9 (MMP-9) and integrin beta 3 (ITGB3) were differentially regulated.

Conclusions

Our study revealed that HEMA even at non-cytotoxic concentrations modulates the expression of numerous genes that are involved in tissue and redox homeostasis. Further experiments are needed to investigate whether these transcriptional alterations translate into modulations on protein level.

P96

The rat femur chamber model for long time observations of implant vascularization and integration in femoral defects

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Autologous bone grafts are still the standard for the regeneration of skeletal critical size defects, as they are osteoconductive and histocompatible. Tissue engineering (TE)-constructs for the reconstruction of bone defects by utilization of different combinations of scaffolds, osteogenic cells and growth factors are an alternative. TE-constructs are size-limited, because their lack of a vascular system causes a severe shortage in nutrition and oxygen supply. To evaluate TE-constructs animal models, which allow the observation of osseointegration and vascular growth are mandatory. One option is the observation via μ CT, which helps to determine bone regeneration inside the constructs, but fails to investigate vascularization of the implant, which is the most important factor for the survival of the implant. Skin fold chambers are ideal for testing TE-constructs over a long time period, as they allow the determination of vascular ingrowth, but they are restricted to the interaction of soft tissue with construct and therefore of low significance for bone TE. The rat femur chamber is a new model to observe inflammatory and vascularization, allowing the evaluation of implants in critical size defects. The chamber serves as a fixation device for the femoral defect and allows the observation of the growing vasculature by utilization of intravital microscopy. We showed that it is possible to observe the neovascularization of nickel-titanium and β -TCP based implants in a femoral defect for at least 20 days. By contrasting the blood plasma with FITC-dextran and leucocytes with rhodamine 6G we measured blood flow, vessel diameter and the inflammatory process. The rat femur chamber is a new model for long time observation of vascularization and inflammation processes after transplantation of new materials as bone substitutes in a critical size defect. As it allows for the repetitive observation of the parameters in one animal, it further reduces the required amount of animals.

P97

Alternative approach to analyze the foreign body reaction to biomaterials on a molecular level – a new strategy for the reduction of *in vivo* experiments

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Introduction

Natural polymers such as collagen are broadly used as biomaterial. Every biomaterial induces a tissue reaction cascade starting with protein agglomeration that is the basis for material-cell-interactions and the extent of the foreign body reaction (FBR) [1]. Nowadays *in vivo* experiments are necessary to analyze the inflammatory potential and biocompatibility of a biomaterial to prevent exaggerated inflammatory reactions (Fig. 1). Various alternatives to animal testing have been proposed. However there is still no method available that allows a prediction of the inflammatory potential of biomaterials. Herein a new method to understand immune reactions on the molecular level based on affinity capillary electrophoresis (ACE) is presented.

Method

ACE is an excellent extension of the Ligand Binding Assay toolbox, even when charge interactions are involved. When a charged ligand binds to a macromolecule, its charge-to-mass ratio is altered. This can be measured with high precision using electrophoresis (Fig. 2). In our consortium, ACE is applied to study the interactions of polymer-based biomaterials to immune-relevant substances such as collagenases, matrix-metalloproteases (MMPs), effectors from the clotting system (fibrinogen) and other molecules.

Conclusion

The present poster introduces the application of ACE as a favorable method for the characterization of protein-protein-interactions, which allows for analysis of the molecular basis of the FBR. This method is proposed to be an optimal tool to reduce animal experiments in the future.

[1] Anderson JM et al. Foreign body reaction to biomaterials. *Semin Immunol* 2008;20:86–100.

Figure 1. TEM image shows a multinucleated giant cell (MNGC) that is phagocytosing fragments of a collagen membrane (Co).

Figure 2 shows the results of a typical ACE experiment. The interaction with ligands shifts the electrophoretic mobility of a binding partner, e.g. a protein, which can be measured very precisely.

Figure 1

Figure 2

Novel imaging techniques to connect performance and assessment of Mg-based implants *in vivo*

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This *in vivo* project investigates the biocompatibility and induced inflammation of degradable magnesium-based implants (Mg-pure and Mg-10Gd) utilizing novel functionalized liposomes as tool for the *in vivo* imaging of biological responses to biomaterials.

In a mice model (B6(C)/Rj-Tyrc/c mice 10 weeks old, female and male, Janvier, Belgium), pins (0.4 mm Ø; 0.5 mm length) were applied to the mouse leg muscle. Sham operation and Ti (grade 5 Ti6Al4V, Ankuro GmbH, Rostock, D) implants of similar geometry were used as controls. The tissue reaction was studied by using the fluorescence indicator MMPsense 645 FAST (PerkinElmer, Waltham, USA) and the novel inflammation sensing liposomes (MESL) developed at MOIN CC. The MESL containing sphingomyelinase (SM) with integrated iron particles and loaded with a fluorescence dye were designed to detect the inflammatory reactions by visualizing apoptosis in the body. Imaging of inflammatory reactions were followed for up to 42 days. The degradation of implant materials was analyzed *in vivo* by μ CT (VivaCT 80 micro-CT, Scanco Medics AG, Brüttisellen, Ch) and *ex vivo* by microbeam X-ray fluorescence spectrometry (μ XRF, Bruker nano, Berlin, D).

The MESL highlighted an initial inflammation reaction for all materials as well as for the controls. However, these signals decreased to the background level until 42 days. Neither increased cell apoptosis nor proliferation was measured. However, an increased collagen synthesis around the implantation area of Mg-10Gd pins was determined. The analysis of embedded muscle samples by μ XRF indicated an enrichment of Ca and P in association with Gd.

The promising results showed the feasibility of the novel MESL for *in vivo* imaging of responses to biodegradable implants. Further *in vivo* studies will be performed in order to clarify the initial inflammation reaction and additional parameter such as molecular indicators for mineralization will be included to aver the biocompatibility of Mg-based implants.

P100

Vergleichende in vitro Biokompatibilitätsuntersuchungen von ästhetischen und konventionellen NiTi-Bögen an primären humanen Gingivaepithelzellen

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In der Kieferorthopädie werden immer mehr ästhetische Apparaturen entwickelt, welche die gleichen Anforderungen an den Behandlungserfolg stellen wie herkömmliche Produkte. Die große Auswahl an diesen Produkten begrenzt sich nicht nur auf Brackets, sondern auch ein breites Spektrum an Bögen, deren Oberfläche mit weißen bzw. zahnfarbenen Materialien beschichtet ist, werden angeboten. Zielstellung dieser Studie war es, die *in vitro* Biokompatibilität diverser Beschichtungen ästhetischer Drähte zu untersuchen. Dazu wurden diese und herkömmliche NiTi-Bögen (Vierkant mit 0.016x0.022") verschiedener Firmen für 14 Tage mit primären humanen Gingivaepithelzellen inkubiert und mittels Cytotox-Glo-Assay die Vitalität und Zytotoxizität der Zellen ermittelt. Als Kontrolle dienten unbehandelte Zellen. Um die Haltbarkeit der Beschichtungen zu überprüfen, wurden die NiTi-Bögen für 6 Wochen klinisch eingesetzt und anschließend der Anteil der verbliebenen Beschichtung ausgemessen.

Bei den ästhetischen Bögen der Firmen American Orthodontics, Dentaureum, World class ortho sowie TP Orthodontics war im Vergleich zu den unbehandelten Zellen die Anzahl der toten Zellen signifikant erhöht. Dabei zeigte der ästhetischen Bogen Everwhite (American Orthodontics) als einziger einen Anstieg der Zytotoxizität im Vergleich zum unbeschichteten NiTi-Bogen. Keine Beeinflussung der Biokompatibilität konnte hingegen bei den ästhetischen Bögen der Firmen GC Orthodontics, Forestadent, Teledenta, Ortho Technology und Dentalline festgestellt werden. Auffallend war, dass die Abnutzung der verschiedenen Beschichtungen nach dem 6-wöchigen klinischen Einsetzen sehr unterschiedlich war.

In der vorliegenden *in vitro* Studie konnte eine Veränderung der Biokompatibilität elastischer Drähte abhängig von deren Beschichtung beobachtet werden. Bei allen Probanden konnten keinerlei Symptome der Unverträglichkeit nach dem klinischen Einsetzen der getesteten Bögen festgestellt werden.

P101

Lasermikrotomschnitte im histologischen Vergleich zu Trenndünnschliff-Präparaten

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Die Beurteilung der Knochenregeneration und der periimplantären Knochenapposition erfordert Labortechniken, die die Beurteilung von Hartgewebe ermöglichen, ohne dieses entkalken zu müssen. Es ist bekannt, dass mit der Trenndünnschlifftechnik 5-15 µm dicke Schnitte präpariert werden können, deren Herstellung fordert jedoch einen hohen Materialverlust ($\geq 0,5$ mm) zwischen zwei Schnitten und jahrelange Ausbildung und Erfahrung. Mit der Entwicklung des Lasermikrotoms ist es möglich geworden, unentkalkten Knochen ohne großen Probenmaterialverlust zu schneiden. Viele wissenschaftliche Publikationen befassen sich mit den Anwendungsmöglichkeiten der einzelnen Methoden. Bisher gibt es jedoch keine Vergleichsarbeit zwischen der Trenndünnschlifftechnik und der Lasermikrotomie. Aus diesem Grund wurden neue Gewebeschnitte mittels Lasermikrotom präpariert und histologisch analysiert. Dabei handelt es sich um Proben, die bereits durch Trenndünnschlifftechnik vorher analysiert worden waren [1].

Bei beiden Methoden ist erkennbar, dass die verschiedenen Implantate vollständig von einer Bindegewebsschicht umgeben sind. Vergrößerungen bis zum 20x führen bei den Schliffpräparaten (50-100 µm) zu keinem Informationsgewinn, da keine einzelnen Strukturen identifiziert werden können. Bei Verwendung von Lasermikrotomschnitten (10 µm) sind Osteozyten bereits bei 10x Vergrößerung deutlich sichtbar. Des Weiteren kann die Grenzfläche zwischen Implantat und Knochen eindeutig analysiert werden. Dabei kann zwischen einer Kapsel und Bindegewebe unterschieden werden.

Auf histologischer Ebene sind Lasermikrotomschnitte nur dann überlegen, wenn durch Trenndünnschlifftechnik dicke Präparate (≥ 30 µm) hergestellt werden. Die Lasermikrotomie besitzt aber darüber hinaus viele Vorteile wie z.B. Zeitersparnis, wenig bis kein Probenverlust sowie gewebeschonende Herstellung von Serienschnitten.

[1] Mai R. et al. Ann Anat 2012, 194: 561-566, Gredes T. et al. Ann Anat 2015, 199: 36-42

P102

Using a graft copolymer to form bioactive implants for bridging complex tissue transitions

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Implants consisting of biocompatible and biodegradable materials are a promising approach towards temporarily replacing endogenous functions and improving tissue healing. Electrospun fiber mats offer various advantages as an implant, such as high surface to volume ratio and good surface roughness and hence can be used to engineer tendon/ligament-to-bone interfaces [1]. For this application, the use of polycaprolactone (PCL) is favorable because of its good mechanical properties and degradation *in vivo*. However, due to its hydrophobicity and lack of easy modification PCL causes several problems regarding cell attachment and growth. Multiple approaches such as chemical modification or plasma treatment were made to improve the properties of PCL. We present another approach using Chitosan-PCL graft copolymers (CS-g-PCL) to introduce surface charges and to improve the cellular behavior [1, 2].

Fig.1: SEM-image of a PCL fiber mat modified with CS-g-PCL copolymer

The modification of electrospun PCL fiber mats using the CS-g-PCL copolymer is an easy and scalable process, which is applicable also to complex structures. Additionally the modification allows further functionalization with nanoparticulate drug delivery systems by electrostatic adsorption. The attachment and homogeneous coating was proven by CLSM employing fluorescently labeled nanoparticle suspensions [3]. Ciprofloxacin has been encapsulated in nanoparticles, attached to the modified PCL fiber mats and subsequently released. First *in-vitro* and *in-vivo* studies showed that our modifications improve biological performance and ingrowth of the scaffolds.

References

- [1] Jing et al., *ACS Appl. Mater. Interfaces*, **2015**, 7 (12), pp 6955–6965
- [2] de Cassan et al, *Colloids Surf. B Biointerfaces* **2018**, 163, 309-320; DOI: 10.1016/j.colsurfb.2017.12.050

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Figure 1

P103

Controlled growth factor release of BMP-2 and TGF- β_3 by nanoparticulate multilayer systems for graded Implants

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Introduction

Functional implants between complex tissues e.g. at a bone-tendon-junction are still an unsolved medical problem. Spatial gradients along the implant could improve the situation. Besides gradients in the mechanical properties, the release of growth factor proteins in a graded way would be a significant progress. For generation of such gradients a combination of nanoparticles as a drug carrier immobilized by the Layer-by-Layer method (LbL) is focussed [1]. Our approach channels on the incorporation of proteins, such as bone morphogenetic protein 2 (BMP-2) or transforming growth factor beta 3 (TGF- β_3).

Materials and Methods

Nanoparticles were prepared from aqueous solution by ionotropic gelation of chitosan and tripolyphosphate or alginate and Poly-L-lysine. The resulting nanoparticles were characterized by particle diameter and zeta-potential. Multilayer films by dip robot were investigated by ellipsometry, AFM and scanning electron microscopy (SEM).

Results and Discussion

Nanoparticles with a well-defined particle size and zeta potential have been obtained after particle formation. The cationic nanoparticles were coated on silicon as a model surface by LbL-technique with different polyanionic interlayers. A linear increase in layer thickness with the number of dipping cycles is observed [3]. By Transferring the multilayer system to modified PCL fibers the construction of multilayers is also possible [2]. BMP-2 and TGF- β_3 were successfully incorporated with an efficiency over 90%. The release from particles and multilayer systems on PCL fiber mats was investigated by an ELISA.

Fig 1.: CLSM image of coated CS-TPP-NPs on PCL-fibermat

Conclusion

Combination of signal protein release from nanoparticles with the LbL technique is a promising method to create a release profile with spatial gradients.

[1] Poth N. et al., *Biomolecules*, 5:3-19, 2015.

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[3] Sydow S. et al., submitted.

Figure 1

P104

Rotator cuff tear repair in a small animal model

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Despite different therapies for chronic rotator cuff tears, re-tears occur regularly. New approaches and implant materials, which aim to reproduce the biological environment, are essentially required.

In a first step, we established in a rat model the surgical, biomechanical and histological techniques for testing new implant materials. In a second step, we used either commercially available porous polyurethane mats or electrospun polycaprolactone (PCL) fibre mats coated with a chitosan-PCL graft copolymer and compared these implants with only suture-fixed tendons.

Fifty-one rats were divided into three groups. In a first surgery, the left infraspinatus tendons were detached and the animals recovered for four weeks. In a second surgery, in 17 animals tendons were fixed with suture material only (control group) whereas in the two experimental groups (17 animals each), the tendons were fixed with suture material and the polyurethane mats or the modified electrospun PCL fibre mats, respectively. The unaffected right infraspinatus tendons were used as intact control. After a recovery of eight weeks, all animals were clinically inconspicuous. Twelve animals of each group were biomechanically tested for stress at failure, stiffness and modulus of elasticity and five animals were analyzed histologically.

Biomechanically, all parameters did not differ statistically between the three groups. However, with respect to the force of failure [N], the median values of the two implant groups were smaller than the median value of the refixed-only group. Histologically, both groups showed no acute inflammation processes, a good infiltration of the mats with cells, ingrowth of blood vessels and tendinous tissue and a normal fibrous ensheathment.

Further investigations should be directed towards enhancement of biomechanical properties and pore size within the electrospun PCL fibre mats to further improve cellular and vascular ingrowth.

P105

Autologous disk cell transplantation - a therapeutic option for lumbar intervertebral disk degeneration. 2 years clinical results of a prospective, randomized, controlled study.

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Intervertebral disc degeneration is in 40% of younger individuals and in more than 90% of individuals older than 50 years of age a common disorder with a negative impact of life quality. Available treatment options, conservative as well as operative procedures, are limited and doesn't treat the underlying biological reason, the intervertebral disc degeneration.

The goals of regenerative medicine at this time are to prevent further progression of disc degeneration and its associated symptoms.

In our clinical trial, with more than 140 patients the largest number of patients with degenerated intervertebral disc disease treated with cell transplantation under defined study conditions, all patients have benefited from transplantation in reduced back pain as one measure of an enhanced quality of life and were able to return to work after transplantation. Moreover a reduction in reherniation rate was more than 50% better than the control group.

The use of autologous chondrocytes requires the ex vivo expansion of cells, adding burdens of cost, time, and regulation that adds to the intricacy of the procedure beyond the medical interface. This limitation of the procedure could be avoided by the use of a one-step procedure, using stem cells obtained from autologous adipose tissue, with shows promising results in preclinical studies.

Cell transplantation appears to present an effective treatment option in degenerative disc disease. The transplantation of autologous disc cells is currently the only biological treatment option with good clinical results under defined study conditions.

P106

Experimental investigation of metal transfer on retrieved femoral heads made of ceramic and metal

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Several studies reported metallic transfer on surface of retrieved femoral heads. In this study, the presence of metallic transfer on femoral heads made of different materials (ceramic, metal and TiN-coated) was compared and the surface topography analyzed. The influence of metallic transfer on the abrasive wear behavior of retrieved heads combined with UHMWPE-liners in a hip wear simulator test was evaluated.

The surface roughness of retrieved femoral heads made of Al₂O₃, CoCr and CoCr with TiN coated were investigated by laser scanning microscope. The composition of the metallic transfer were determined with EDX analysis. Furthermore, retrieved ceramic heads with 28mm (n=3) and 36mm diameter (n=3) with extensive metallic transfer were tested with brand-new UHMWPE-liners loaded for 5 million cycles in a hip wear simulator according to ISO 14242-1. The abrasion of the PE liners was determined gravimetrically. The expansion of worn area on heads was determined before and after the abrasion test with digital microscope.

On all examined femoral heads metal transfer could be proven. These areas resulted in a higher surface roughness compared to unconcerned areas. In EDX analysis, mainly titanium and cobalt-chromium were identified as transferred materials. In the gravimetric wear measurement of the UHMWPE-liners, a wear rate of 7.35 ± 1.77 mg per million cycles was determined for 28mm ceramic heads and 9.72 ± 1.92 mg per million cycles for 36mm ceramic heads. After 5 million cycles in simulator, a reduction of the metal transfer on the retrieved heads was determined.

In this study, metallic transfer on retrieved ceramic heads and first also on heads made of CoCr and TiN coated CoCr with increased surface roughness could be detected. The wear rate of UHMWPE-liners was five times higher articulating with retrieved ceramic heads compared to brand-new ceramic heads. To minimize wear in artificial joint, any contact femoral head with metallic acetabular cup should be prevented.

P107

The in vivo cellular reaction towards biomaterials: Integration and disintegration and their consequences

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Introduction

Biomaterials are widely used for the regeneration of bone and soft tissue in different indication fields of oral and maxillofacial surgery. However, it is still unclear which biomaterial to choose for which indication. After biomaterial application the cellular reaction is of great importance for the regeneration process. This study series evaluated different biomaterials in vivo to evaluate the cellular reaction of different biomaterials based on their specific physicochemical properties.

Materials and methods

In a translational studies series, the cellular reaction towards different biomaterials was histologically analyzed using preclinical implantation models in mice, rats and rabbit. Biopsies including clinically applicable biomaterials were gained during clinical trials and analyzed histologically.

Results

Histological analysis showed mainly two different cellular reactions. Biomaterials that induced only mononuclear cells were characterized as "physiological reaction" including lymphocytes, monocytes and macrophages. These biomaterials underwent integration without premature breakdown. The second class included biomaterials that induced a high inflammatory reaction including multinucleated giant cells (MNGCs). This group was characterized as "pathological reaction". These biomaterials underwent rapid degradation and were disintegrated from the implantation area. The induction of MNGCs was ubiquitously and independent of the species. Biomaterial induced MNGCs showed morphological similarities to pathological MNGCs induced by inflammatory diseases such as tuberculosis and expressed high proinflammatory signaling molecules.

Conclusion

The present translational study series demonstrated the importance of the cellular reaction of biomaterials and their consequences for the clinical application to define more tailored clinical indications for biomaterials according to their inflammatory pattern and degradation capacity.