ABSTRACTS

Oral presentation

Session 1

Adding of nanostructures for enhanced bone regeneration.
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Bone regeneration scaffolds are mainly used to support bone regeneration after severe bone fractures, advanced osteoporosis, removal of bone tumours, craniomaxillofacial surgery, and several dental/periodontal indications. Our approach to apply nanotechnology to modify the polymer and ceramic scaffold surface so that osteoblast proliferation is enhanced and bone ingrowth accelerated is presented. Two kind of porous scaffolds (polymer and ceramic) were prepared and nanustructured calcium phosphate coating was applied and successful in vitro and in vivo results have been achieved. The challenge was to coat all the internal surfaces of a porous scaffold with a resorbable nanostructured hybrid layer that improve biocompatibility and the new bone ingrowth rate into the pores of biodegradable polymer and ceramic scaffolds.

The influence of porosity on the fatigue properties of brushite cement
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There are several different synthetic bone grafting materials that can be used as injectable bone void fillers and to stabilize fractures together with hardware. Calcium phosphate cements (CPCs) provide one such alternative, but their use is partly limited by the lack of knowledge of their mechanical properties over longer periods of time, in particular the fatigue properties. This is unfortunate since when implanted in vivo the cement will experience dynamic loading. Recent studies have shown clinical advantages of acidic cements compared to the commonly studied basic apatite cements, in terms of faster resorption rates [1,2] and osteoinductive effects [3], permitting faster bone regrowth. The aim of this study was to investigate the relationship between cement porosity and the compressive fatigue behaviour of a strong brushite cement. The porosity of the cement was varied by using three different liquid to powder ratios and evaluated by means of a solvent exchange method and micro computed tomography. The compressive fatigue properties of the cements were studied using stress levels between 15-30 MPa, at 20 Hz and using 5 million cycles as run-out limit. The results suggest that both the cyclic fatigue life as well as the quasi-static compression properties depend on the total porosity of the cements. No apparent relationship between the largest pore size and the cyclic fatigue life of the samples could be found. In conclusion, brushite cements show promising results in terms of fatigue performance since the stress levels the material can sustain is in the range of reported compressive strengths of human trabecular bone.
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Session 2

Vascularization of Multi-Organ-Chips for Tissue Engineering and Regenerative Medicine
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Organ-on-a-chips provide an adequate and reliable system for drug testing and engineering of small tissues. Yet, a big drawback is the lack of an intrinsic vascularization of tissue-engineered constructs. Here, we demonstrate the incorporation of fibrin scaffolds with endothelial and adipose-derived stem cells embedded into a perfused

closed multi-organ-chip (MOC) system. We show that under static conditions vascular network formation is influenced by both fibrinogen and thrombin concentrations. 3D-rendered images using two-photon microscopy analysis show organized microvascular structures in samples cultured under static or flow conditions for two weeks. We furthermore report that the use of serum- and growth factor-free basal media did not impair the maintenance of formed vascular structures when the media switch occurs on day 4 of incubation. Moreover, using fluorescencelabeled fibrinogen we monitored fibrin degradation under static conditions and address the use of the fibrinolysis inhibitor aprotinin for optimal microvessel formation. Finally, we conclude that a vascular network can be established by co-culturing endothelial and adipose-derived stem cells in a perfused organ-on-a-chip model. Further experiments will address whether additional layers of microtissues can be vascularized by this system.

Coating kinetics of bioinspired phenolic films on titanium surfaces

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Introduction

Polymeric coatings which can be applied to virtually any kind of material have gained increasing attention over the last years.[1] Among them, (poly)phenols have recently been found to form material-independent self-polymerizing coatings with antibacterial and antioxidant properties.[2] The aim of this study was to gain knowledge about the coating kinetics of two selected (poly)phenols on titanium surfaces.

Experimental

The coating kinetics of tannic acid (TA) and pyrogallol (PG) was investigated in real time by QCM-D and ellipsometry. The chemical composition of the coatings was examined by XPS and FTIR.

Results and discussion

The formation of TA coatings was characterized by three phases. First, decreasing frequency and low dissipation displayed the build-up of a rigid layer. After ~2 h, the layer grew in a more viscoelastic manner represented by strong increase in dissipation with continuous decrease in frequency. Finally, the frequency reached a constant value after 4-5 h while dissipation kept increasing. Ellipsometry confirmed these observations with coating thicknesses reaching a plateau at ~50 nm after 4 h.

In contrast, PG showed the deposition of a dissipative initial coating followed by the growth of a more rigid layer after ~6 h. The layer growth was continuous reaching thicknesses of ~75 nm after 24 h. The phenolic composition of the coatings was confirmed by XPS and FTIR.

Conclusion

The two (poly)phenolic systems behaved differently regarding the kinetics of the coating formation. TA showed a multiphasic and fast layer build-up. In the later phases, PG displayed a more stable and predictable growth of the coating layer, making the application of the system better controlled.

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Titanium surface modification leading to increased antibacterial ability, bioactivity and biocompatibility

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Introduction

One way to prevent bacterial biofilm formation on implants is to modify the surface to make it antibacterial or bacteriostatic. A straightforward and economically attractive modification method is to soak the surface in hydrogen peroxide, sodium hydroxide and calcium dihyroxide. Hydrogen peroxide has been found to make the surface antiinflammatory[1] and perhaps antibacterial. Sodium hydroxide has been seen to render the surface bioactive[2], while calcium hydroxide might further increase the bioactivity. In this study we investigated the antibacterial properties, bioactivity and biocompatibility of this surface modification method.

Methods

Coupons of cpTi (grade 2) were immersed in H_2O_2 for 1 h at 80 °C and then soaked in NaOH. Some test groups were also soaked in $Ca(OH)_2$. After soaking, coupons were heat treated at 200 °C, autoclaved at 125 °C for 1 h or simply kept at room temperature. To investigate bioactivity the coupons were immersed in SBF for 7 days. Biocompatibility was assessed by seeding two cell lines on the modified titanium surfaces. To investigate the antibacterial effect, bacterial biofilms were grown on the surfaces for 16 h and assessed for viability with luminescence readings. Results

$Ca(OH)_2$ modified coupons showed an increased bioactivity compared to coupons only soaked in NaOH.

Hydroxyapatite formation was strongest for test groups placed in room temperature or 200 °C. Cell proliferation was increased with human dermal fibroblast. Autoclaved surfaces showed a decreased luminescence signal compared to the control, indicating inhibition of bacterial biofilm.
Conclusions
Coupons soaked in $Ca(OH)_2$ after soaking in NaOH showed increased bioactivity compared to coupons only soaked in NaOH. Further they exhibit excellent biocompatibility and some degree of antibacterial behavior.
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Poly(trimethylene carbonate) degradation by macrophages is modulated by inflammation-interfering agents
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Poly(trimethylene carbonate) (pTMC) is a flexible biodegradable polymer that can be degraded by macrophages *in vitro*. The degradation depends on macrophage activity, presumably by the use of cholesterol esterase and reactive oxygen species. Therefore it is likely that agents modulating inflammatory processes also interfere with macrophage activity towards pTMC degradation.
In this study J774 mouse macrophages were subjected to treatment with LPS, LTA, IFN-y, LPS + IFN-y, fibroblastconditioned medium, indomethacin and methyl-β-cyclodextrin. Results included the cell number, the pTMC weight loss, the XTT metabolic activity, nuclear (DAPI) and actin cytoskeleton (by phalloidin) staining with confocal laser scanning microscopic evaluation, ROS formation and cytokine determinations. Every second day the macrophagemediated degradation and associated macrophage response was assessed up till 6 days of incubation. Also fresh macrophages were given every second day.
Statistic differences were found between groups. The indomethacin, control and fibroblast conditioned medium exposed macrophages showed the most degradation of pTMC, while the IFN-y + LPS group showed the most degradation of pTMC per macrophage, but the least absolute degradation. Furthermore, it was found that there is neither relation between the cell activity and degradation of PTMC nor a relation between the amount of macrophages and degradation of PTMC. Indomethacin significantly stimulated the ROS production of the macrophages.
The findings are discussed in relation to the modulating effects of the compounds investigated with respect to the different forms of macrophage activities, including those related to pTMC degradation. For example, the finding that indomethacin has such a profound effect on macrophage-mediated pTMC degradation and ROS activity is difficult to match with the anti-inflammatory action of indomethacin.

Session 3

Design of a New Dual Hernia Mesh with an Absorbable Nanofibrous Layer
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Dual meshes are preferred to use in the case of umbilical and incisional hernias where the abdominal wall defect is very large. These meshes can be composed of two layers from non-absorbable materials or one layer may be from absorbable that has ability to degrade after healing of the wall. The most crucial point in the design of a composite mesh is to produce two mesh layers with different properties. One layer would serve as a non-adhesive barrier for viscera while the other supports the restoration of abdominal wall.
The main propose of the entire study is to design a novel dual layer composite mesh for the treatment of abdominal hernias. The developed mesh consists of two layer; one is a nanofibrous layer made of poly(glycerol sebacate)/poly(caprolactone) to support the healing of abdominal wall defect, while the other layer has non-adhesive properties to avoid the viscera adhesion to the mesh. A smooth film was produced from a medical grade polycarbonateurethane by either a simple solvent casting or a compression moulding process. For the adhesive layer, the PGS pre-polymer was synthesized by polycondensation and characterized by different methods, namely NMR, FTIR, DSC and GPC. Then PGS/PCL mixture was electrospun to obtain nanofibrous membrane which would glued onto PU film afterward. The mechanical analysis of the final products revealed that developed meshes may compete with the commercial products. Regarding to *in vitro* degradation studies, nanofibrous layer have already lost 25% of its weight after 30 days in PBS. SEM images also confirmed the degradation of nanofibers which was mainly related to PGS. It is expected that the developed meshes can be used practically in clinical applications after *in vitro* cell culture and *in vivo* animal studies.
This work was supported by The Scientific and Technological Research Council of Turkey under project 214M134.

New insights into co-culture systems for microcapillary tissue engineering
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Vascularization of engineered constructs is critical for most tissues, yet still remains as one of the great challenges in Tissue Engineering and Regenerative Medicine. Different concepts have been developed to properly vascularize artificial tissues. Besides technical approaches like rapid prototyping, the use of two-photon polymerization or microfluidics, the use of co-cultures of blood vascular and lymphatic endothelial cells and other cell types provides a suitable method to generate microvascular structures. Endothelial cells from different origins like peripheral blood, dermis or umbilical veins have been shown to form perfusable vascular structures in combination with e.g. fibroblasts, osteoblasts or mesenchymal stem cells. Our group combines human endothelial colony forming cells (ECFC) or lymphatic endothelial cells (LEC) with adipose-derived stem cells (ASC) in a fibrin matrix to enable formation of two separate blood vascular and the lymphatic networks. Blood vascular tube formation is partly dependent on the physical interaction with both cell types, as evidenced by analysis of the secretomes from mono- and co-cultures. Furthermore, we work on a completely autologous approach using all cells from one individual and avoiding any animal-derived constituents. E.g. we show the suitability of human platelet lysate (hPL) as a substitute for fetal calf serum (FCS) in the culture of these cells. Ultimately, unravelling the molecular and functional features of these artificially generated vascular structures is a prerequisite for any clinical consideration.

Session 4

Proteome analysis of human serum proteins adsorbed onto different titanium surfaces used in dental implants
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Titanium (Ti) is often utilized in dental implants; blasted acid-etched Ti is used more frequently than smooth Ti surfaces. The deposition of proteins onto the implant surface might affect osseointegration. In this study, both surface types were used to examine the protein layers formed after incubation with human serum. Physicochemical characterisation revealed important differences in roughness, chemical composition and hydrophilicity. Using mass spectrometry (LC/MS/MS), 139 proteins were identified, 31 of which were associated with bone metabolism. Interestingly, Apo E, antithrombin and protein C adsorbed mostly onto blasted and acid-etched Ti, whereas the proteins of the complement system (C3) were found predominantly on smooth Ti surfaces. These results suggest that physicochemical changes could be responsible for the differences in the observed adsorbed protein layer. No differences were found in *in vitro* mineralization studies, suggesting that these proteins do not affect osteoblast differentiation directly but might be related to *in vivo* processes.

Mechanical Properties of Alginate Gels Containing Grafted Alginates
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Alginate is a relevant biopolymer for tissue engineering applications due to its ability to form hydrogels at close to physiological conditions, and its biological inertness. However, the alginate can be made biologically active through grafting of bioactive molecules[1]. The biopolymer is a linear polysaccharide composed of β-D-mannuronic acid (M) and α-L-guluronic acid (G), where mainly G-blocks give alginate its ability to form a gel with divalent cations[2]. The mechanical properties of the alginate gel can be designed by enzymatic[3] and chemical[4] modifications of the alginate. In this study, bioactive compounds were grafted to partially oxidized alginate by reductive amination[5] or by reductive amination and cupper-catalyzed azide-alkyne click-chemistry. Calcium cross-linked gels were made of alginate with a high G-content mixed with the grafted alginate, and mechanical properties were determined. Generally, the gels containing the coupled material showed a decrease in compression strength and stability compared to the gels where no coupled alginate was mixed in. This can be explained by a reduction in crosslinks in the gel due to a destruction of the G-blocks upon grafting. Hence,

alginate gels with desired mechanical properties and bioactivity can be tailored through the mixing in of grafted alginate with non-grafted alginate.

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Session 5

Calcium phosphate-alginate composite materials as soft scaffolds for hard tissue

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The desired functions of biomaterials have over the last decade shifted from inert to bioactive and instructive. At the same time there has also been a drive towards 3D culturing of cells as better models of their natural environment. Hydrogels can provide a matrix for cells and may be loaded with different bioactive agents, making them a promising candidate for this new generation of biomaterials.
We have previously developed a hydroxyapatite-alginate composite material which has shown potential as a bone tissue engineering scaffold. The literature suggests that the more soluble calcium phosphate (CaP) phases may provide faster resorption *in vivo*, mainly due to dissolution, and in some cases even exhibit osteinductive behavior. Recent work has been focused on fabrication and characterization of CaP-alginate composites with more the soluble brushite as the inorganic component. Precipitation of brushite within the alginate gel was achieved by incorporating brushite seeds in the alginate prior to gelation and reducing the pH to increase the stability of the mineral. This fabrication protocol could possibly reduce the viability of encapsulated cells, however, it was shown that pre-osteoblast cells survived well inside the mineralized gel for a period of 15 days. In addition, the mineral transformation into HAp upon *in vitro* storage in simulated body fluid has been studied.
In this contribution I will show how a combination of optical microscopy, confocal laser scanning microscopy, Raman microspectroscopy, electron microscopy and micro-X-ray fluorescence spectroscopy can be employed to extensively study the formation of mineral, such as CaP, inside a hydrogel matrix, in this case alginate. This approach enables the extraction of mineralization and gelation kinetics, localized pH and chemical composition as well as mineral distribution and morphology. This information provides an excellent platform for further development and optimization of hydrogel based composite materials.

New graphene oxide reinforced gelatin-poly(vinyl alcohol) porous scaffolds as biomaterials

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Introduction
One of the main contributors to biomaterials development in application sectors such as wound dressing / healing, drug delivery systems, tissue engineered scaffolds, *etc* is mirrored by polymers science. Whilst biopolymers in both natural and synthetic forms present their advantages and disadvantages, their combination into composite materials is a useful strategy to ensure superior features. In this manner, the present study reports the fabrication of new graphene oxide reinforced gelatin-poly(vinyl alcohol) (Gel-PVA/GO) porous scaffolds by coupling freeze-thawing with freeze-drying techniques. Materials were characterized by Fourier transform infrared spectrometry (FT-IR), Xray diffraction (XRD), transmission electron microscopy (TEM), X-ray microtomography (microCT) and scanning electron microscopy (SEM). Nonetheless, mechanical tests against compressive stress, as well as biocompatibility tests, were performed. **Results**
Structural analysis by FT-IR, XRD and TEM revealed interactions between GO nanosheets and the two polymers, which resulted in a unique molecular structuration. Further on, both microCT and SEM emphasized on the influence of GO in adjusting pores size and shape. Mechanical tests measurements showed an improvement of materials' compressive strengths by 97 - 100 % with the addition of 0.5 - 3 wt% GO. Eventually, cytotoxicity and cells viability assessments suggested Gel-PVA/GO composite scaffolds met the requirements for further *in vivo* testing and tissue engineering applications. **Conclusions**
New Gel-PVA/GO biocomposite scaffolds were prepared and characterized in terms of structure, morphology, mechanical performances and biological activity. Results indicated the GO beneficial effect on the polymer matrix, especially in consideration of mechanical resistance and biological assessments, which recommended

Gel-PVA/GO composites for further *in vivo* testing and might open new prospects for the development of tissue engineering applications.

Acknowledgements
This study was funded by a grant of the Romanian National Authority for Scientific Research, Executive Agency for Higher Education, Research, Development and Innovation; project number PN-II-PCCA-140/2012, EEA Grants project number 12/12.07.2014.

Rapid production of sol-gel and porous inorganic-organic hybrid fibres via solution blow spinning

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Solution blow spinning (SBS) produces micro- and nanofibres, analogous to those electrospun, from polymers dissolved in suitable solvents, yet with a production rate circa 100 times more rapid than electrospinning and without the need for electric fields. SBS uses a system of concentric nozzles, with precursor solutions blown and stretched to form fibres, using pressurised air.
In this work we extend the technique to sol-gel processing to create both inorganic and inorganic-organic hybrid fibres, which have tailorable degradation rates, mechanical properties and ion release mechanisms. Formulations were designed based on tetraethyl orthosilicate (TEOS) as the sol-gel precursor. TEOS was first hydrolysed, and allowed to polycondense until reaching a viscosity suitable for spinning into fibres. The precursor was injected at 200 µl/min via a syringe driver and air pressure of 30 psi applied for SBS. Fibres were collected at a distance of 20 cm, sufficient to evaporate the solvent phase. Hybrids were produced by adding 10 wt.% of gelatin in water to the hydrolysed TEOS solutions. Selected hybrid solutions were blow spun both directly into a cryogenic bath of liquid nitrogen, to produce porous fibres. On contact with the liquid nitrogen the fibres are vitrified and undergo thermally induced phase separation, and are then subsequently freeze-dried. The time, and rate dependent rheological properties were measured and correlated against spinnability. Fibre diameters and morphologies were assessed via scanning electron microscopy and image analysis (ImageJ). The inorganic content was determined by
thermogravimetric analysis. Neat sol-gel silica fibres of smooth morphology and average diameter 1.7 ± 0.8 µm were produced. The silica-gelatin hybrid formulation spun at room temperature resulted in fibres of average diameter 2.2 ± 0.5 µm, with a rugose nano-scale topology. Cryogenically collected silica-gelatin hybrid fibres were of larger diameters (~ 4 µm) and exhibited highly porous interiors.

3D Printed Anisotropic and Hierarchical Bone Inspired Nanocomposites

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Introduction: Our research focuses on developing bone-mimetic nanocomposites for orthopedic applications. Today, the average lifetime of a hip implant is 5 years. The major issues causing failure is tissue rejection, mechanical mismatch between implant and tissue and infection. In light of this, we aim to develop implants based on biocompatible, synthetic materials inspired from bone to help regenerate the natural tissue.
Methods: We have developed a polymer-calcium phosphate (CaP) composite that mimics the ordered nanostructure and CaP chemistry of the collagen-calcium phosphate assembly in bone. Specifically, based on a bottom up, molecular self-assembly where we use polymerized liquid crystals (LC) with ordered mesoscopic domains to host the formation of well-distributed inorganic CaP nanoparticles[1]. This results in a highly ordered, fibrillar and organic-inorganic interpenetrating nanocomposite much similar to that observed in bone.
Results: Analysis of the nanocomposite using electron microscopy (SEM and TEM) and X-ray diffraction (XRD and SAXS), showed that the CaP particles were chemically similar to bone apatite. The composites are mechanically tough and stable over a period of 150 days. To mimic the structural hierarchy and mechanical properties of bone, we are fabricating the bone-mimetic composites using extrusion 3D printing. This technique induces a structural alignment of the LC matrix at the micron to millimeter scale. The alignment of the LCs along the printing direction also drives the formation of CaPs in the same direction resulting in a highly anisotropic material. SAXS scattering and SEM images clearly confirm the alignment of the LCs and consequently the CaP particles along the printing direction.
Conclusion: In this work, we have developed a 3D nanocomposite with unprecedented control over the nano, micro and macro structure and chemistry.
He, W.; Rajasekharan, A. K.; Bagha, A.; Andersson, M., Adv. Mater. 2015, 27, 2260-2264

Session 6

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Functionalization of alginates for tissue engineering applications

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Peptide grafted alginates obtained by chemical functionalization of alginates are attractive as scaffold materials for cells in regenerative medicine and tissue engineering. We here present our latest work on two different strategies to produce peptide grafted alginates[1,2]. Carbodiimide chemistry with substitution on the carboxylic group is the most commonly used strategy. We have used a chemo-enzymatic method by first grafting of mannuronan with subsequent epimerization[1]. The obtained degrees of substitution were low (0.1 - 0.2 % of carboxylic groups grafted with peptide). However positive effect on attachment of mouse myoblasts (C2C12) and primary rat olfactory ensheating cells were seen. In the second strategy, partial periodate oxidation followed by reductive amination is presented as an attractive alternative for grafting[2]. High and precise degrees of substitution were obtained with high reproducibility, and without formation of by-products. A protocol was established using the non-toxic pic-BH_3 as the reducing agent. Covalent binding was indirectly verified by DOSY and the structure of the product was further elucidated using NMR spectroscopy. The coupling efficiency was to some extent dependent on alginate composition, being most efficient on mannuronan. Three different bioactive peptide sequences (GRGDYP, GRGDSP and KHIFSDDSSE) were coupled to 8 % periodate oxidized alginate resulting in degrees of substitution between 3.9 and 6.9 %. Cell adhesion studies of mouse myoblasts (C2C12) and human dental stem cells (RP89) to gels containing various amounts of GRGDSP coupled alginate demonstrated the bioactivity of the material where RP89 cells needed higher peptide concentrations to adhere[2].

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Biodegradable magnesium implants and their influence on cell metabolism

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Aging populations, increasing obesity and a rise in osteoporosis-related fractures will sustain a need for orthopaedic intervention. In addition, juvenile patients and active adults exhibiting risky sporting activities also require perfect care. So far these indications are treated mainly with non-degradable metal implants or in some cases also polymers. From the patient's point of view, degradable implants would clearly be preferred. Here degradable Magnesium based implants could become an alternative to permanent metallic implants which have to be removed after healing, or to replace degradable polymers which do not always show the required mechanical properties. Mg and its alloys degrade under physiological conditions. The great challenge here is to tailor the degradation in a manner that is suitable for a biological environment. Fast or uncontrolled corrosion is associated with strong hydrogen and ion release and severe pH changes, which can lead to a fast loss of mechanical stability and undesirable biological reactions[4]. Since these processes are highly complex in a living system and sufficient data describing the degradation *in vivo* is missing, it is very difficult to produce knowledge based new alloys. Therefore the development of new biodegradable Mg-based implants is strongly relying on the understanding of the degradation process in the living organism and the creation of an appropriate test system *in vitro*.

This presentation summarizes the influence Mg has on osteoblast, osteoclasts and macrophages. There are indications that indeed the release of locally high concentrations of Mg can stimulate bone remodelling. This is done in a multiple fashion: proteins responsible for bone matrix formation are strongly expressed but also the cross talk between osteoblasts and osteoclasts is influenced. In addition macrophages are directed into an anti-inflammatory and tissue remodelling pathway.

Antimicrobial peptide immobilization of Elastin-like proteins for use as bioactive antimicrobial surface coatings

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The development of antibacterial surfaces using antimicrobial peptides (AMPs) seems to be a very attractive approach for biomedical applications. Compared to conventional antibiotics, these peptides are shown to have broader spectrum of activity and rarely promote bacterial resistance. Antimicrobial peptides are naturally the innate immune system effectors to defend host organisms against microbes, hence most of them show lower toxicity to mammalian cells.

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However these peptides have relatively short life time and modest antibacterial activity. Covalently bonded to biomaterials surfaces such peptides can acquire long-term stability and retain their bactericidal activities at very low concentrations.
Recombinant elastin protein (ELP) thin films were used for immobilization of antimicrobial peptides. ELP containing RGD sequences can provide favorable cell-adhesive surfaces for *in vivo* interactions. Attaching AMPs to ELP thin films, can provide a new family of bioactive materials with high antibacterial activity.
In this work an antimicrobial peptide (RRPRPRPRPWWWW-NH2) was covalently bonded to substrates coated with ELP and the Bacteria responses to these surfaces were evaluated.
Recombinant elastin proteins (ELP) with RGD sequences and photo reactive sites were expressed in *E-Coli* culture followed by purification. Uniform ELP thin films were formed by spin coating, followed by crosslinking with UV radiation. NHS/EDC coupling method was used to covalently bond AMP to ELP surfaces. The surfaces were used to grow *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. The results showed a significant bactericidal effect when the films were functionalized by antimicrobial peptides.
The ease and scalability of producing ELP coatings and Antimicrobial peptides functionalization process, makes these surface modification an ideal candidate for the development of novel antimicrobial surfaces for biomedical applications. **References**
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Pathogen inactivated platelet lysates boost osteogenic and chondrogenic differentiation of MSC and mesenchymal progenitor cells
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INTRODUCTION: The suitability of mesenchymal stromal cells (MSC) for therapies has been studied extensively. MSC are particularly interesting due to their multipotent differentiation and immunomodulatory abilities. Embryonic derived mesenchymal progenitor cells (hES-MP) have the same characteristics as MSC and have been thought of as a cell line that can be used therapeutically as MSC. Addition of animal-serum is currently required for the survival and growth of hES-MP and MSC. Using animal-serum to grow cells intended for human medical therapy is not safe and serum-free alternatives need to be found. We have previously demonstrated the efficacy of using platelet lysates from pathogen inactivated platelets on MSC and hES-MP during their growth. Here we show that using human platelet lysates made from pathogen inactivated platelets (hPL-PI) during differentiation of MSC and hES-MP fully supports both chondrogenesis and osteogenesis allowing serum-free growth conditions from expansion throughout differentiation.
METHODS: MSC and hES-MP002.5 were grown and differentiated towards chondrogenic and osteogenic lineages in the presence of fetal bovine serum (FBS) or hPL-PI. For chondrogenic differentiation, histology and gene expression was evaluated over five weeks. For osteogenic differentiation alkaline phosphatase activity, mineralization and gene expression evaluated over four weeks.
RESULTS: Histology resembling articular cartilage was observed for at the end of chondrogenic differentiation when differentiated in the presence of hPL-PI and chondrogenic gene expression was significantly upregulated. During osteogenic differentiation the activity of alkaline phosphatase, amount of calcium deposits and gene expression was significantly higher in MSC and hES-MP differentiated in hPL-PI compared to FBS.
DISCUSSION & CONCLUSIONS: Using hPL-PI based differentiation media boosts osteogenic and chondrogenic differentiation of MSC and hES-MP to a greater degree than FBS based media. Using hPL-PI rather than FBS provides a serum-free environment for the cells. New guidelines advise against the use of animal-serum making hPL-PI an attractive alternative.

Session 7

Engineering physico-chemical microenvironment for stem cell-based regenerative medicine of bone tissue
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When engineering a pseudotissue *in vitro* for subsequent engraftment *in vivo*, a scaffold should provide a structured environment with tissue-specific mechanical properties sustaining the differentiation of stem cells (SCs) seeded therein. Cell expansion and differentiation in culture will also take advantage of using a ‘bioreactor’, i.e. an incubator where a mechanical actuation can precondition the constructs, mimicking the target in vivo

environment. This paper proposes bioactive 3D poly-lactic-co-glycolic acid/alginate scaffolds as an engineered extracellular matrix (ECM) for bone tissue engineering within a novel mechanical actuation system.
3D scaffolds, embedding PLGA microcarriers fabricated via supercritical emulsion extraction (SEE) technology, were developed by cross-linking Ca-Alginate (2% w/w) in 0.1M calcium chloride solution. The SEE apparatus is based on a high pressure packed tower (2000 height; I.D. 10 mm) working with supercritical carbon dioxide (SC-CO_2) flowing (1.4 kg/h and 0.1 L/G ratio) at 8 MPa and 38°C. These scaffolds are intended to support SCs expansion and differentiation within a standalone bioreactor generating perfusion/compression stimuli. This device also monitor culture parameters such as: flow rate, pressure, pH, humidity and temperature. Automatic replacement of the media is incorporated. Characterization of the scaffolds cultured as described was carried out with SEM, x-ray uCT analysis and 3D modeling techniques.
Preliminary results show that these matrices are adequate for a bone structure. Both the mechanical and the biochemical activity of the scaffold is improved by the insertion of uniformly dispersed PLGA microdevices (mean size 0.75 um; standard deviation ± 0.18 um). Indeed, when entrapped within the alginate 3D structure they can a) undergo micro-shift around their position as a consequence of the perfusion/compression stimulus and b) grant the controlled delivery of biochemical cues. All these stimuli are anticipated to enhance SCs differentiation once they are loaded into the scaffold.

Improving mechanical properties of porous bioactive glass scaffolds for bone regeneration with stereocomplex polylactide coatings

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Bioactive glass scaffolds can be favourably used for the regeneration of bone. However, because of the inherent brittleness of glass, polymer/glass composite scaffolds may be better suited for bone regeneration purposes than scaffolds based only on glass, especially in sites where higher mechanical strength is needed. Polylactide (PLA) can exist in amorphous or semicrystalline morphologies. A special form of PLA crystals, called stereocomplexes (scPLA), is known to have higher mechanical strength and slower hydrolytic degradation than amorphous or semicrystalline PLA. The aim of this study was to evaluate the impact of sc-PLA coatings on mechanical and degradation properties of porous bioactive glass scaffolds.
Porous scaffolds of the bioactive glass 13-93 were manufactured with the foam replication method. The glass scaffolds were dip-coated with amorphous poly(D,L-lactide) (PDLLA), semicrystalline poly(L-lactide) (PLLA) or an equimolar mixture of PLLA and poly(D-lactide) (PDLA) for stereocomplex formation. After drying the scaffolds were annealed in order to increase the amount of stereocomplex crystals in the coating. In vitro degradation of the scaffolds in simulated body fluid (SBF) was studied for up to 10 weeks. Compression strength was measured both before and after in vitro degradation. Water uptake, mass loss and pH of the SBF were measured. Pore morphologies were characterized with SEM and μ-CT.
The polymer coating on the glass scaffolds was evenly spread and the coating covered the pore walls to a large extent. Stereocomplex coating yielded the highest compression strength and the strength did not decrease during the 10 week long hydrolysis time. The pH of the immersion medium was higher for stereocomplex coated scaffolds than for the other coatings. This work gives important insight into the applicability of sc-PLA for simple adjustment of the mechanical and degradation properties of scaffolds for tissue regeneration.

Bone material distribution in the femur as an index for pre-surgical planning

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When patients are scheduled for total hip arthroplasty, the decisions with regards to the type of the implant and fixation method are made based upon the expertise of the surgeon. Having quantitative measurements can assist the surgeon in the decision making progress and therefore improve the surgical outcome. Measurements and calculations on the mechanical integrity of the femur can play an important part in determining which fixation method to use, press fitting which relies on the mechanical interactions between the implant and the bone or using cement to fix the implant inside the femoral canal. The bone density distribution defines the mechanical strength of the bone and measurements on the material distribution using CT scans can give indications about how well the bone is equipped to be subjected to the mechanical loading, both during the surgery as well as during activities of daily living post-operatively. In the presented study, patients scheduled for total hip arthroplasty underwent CT scanning alongside a phantom used to calibrate against the material density. Linear relationship was used to connect the pixel values from the scans with the bone mineral density and an empirical formula used to connect the bone mineral density to both the stiffness and the ultimate stress of the bone material. Finite element models were created from the scans and loading conditions applied simulating press fitting procedure and the fracture risk calculated. Results showed that models where increased amount of cortical bone was present, there was less risk of the bone fracturing during the press fitting procedure. Mapping the three

dimensional distribution of the bone material and quantifying the amount of cancellous bone and cortical bone can assist surgeons in the decision making process with regards to implant type and fixation method.

Chitosan-peptide conjugates as effective antimicrobial agents

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Antimicrobial peptides (AMPs) hold great promise as potential biopharmaceutical drugs for the treatment of infections. However, clinical applications of AMPs have to overcome a number of significant challenges, including the toxicity, due to the relative high minimum inhibitory concentration, the low selectivity for bacterial membranes as well as their short half-life in vivo. We envisioned that an improvement in antimicrobial activity could be obtained by coupling of multiple copies of AMP (anoplin) monomers to a linear, biocompatible carbohydrate polymer, chitosan, for multivalent display and at the same time possibly reduce the hemolytic toxicity of the peptide.
Chitosan-peptide conjugates were synthesized through copper-catalyzed azide alkyne cycloaddition. Solid phase synthesis of the peptide anoplin having either an *N*-terminal propargyl group or a *C*-terminal propargyl group was carried out. TBDMS protected chitosan was utilized for synthesizing 2-azidoacetyl chitosan derivatives having varying degree of substitution (DS), which were then coupled to the peptide anoplin through either *N* or *C*-terminus. The compounds were characterized using 1H and COSY-NMR, HSQC, HMBC, IR and CD spectroscopy. The conjugates displayed enhanced antibacterial properties towards *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa* than the peptide anoplin. The highest activity amongst the conjugates was observed towards *Escherichia coli* with an MIC value as low as 4 µg/mL. The *N* and the *C*-terminal conjugates exhibited an increasing order of activity with increasing DS towards *Staphylococcus aureus* and *Escherichia coli*. The *N*-PEP-CS series showed a drop in activity with increasing DS, while the compounds of the *C*PEP-CS series remained independent of the DS variations and showed fixed MIC values towards *Enterococcus faecalis* and *Pseudomonas aeruginosa*. The toxicity of the derivatives towards human red blood cells was found to be significantly reduced (HC_{50} value = ≥ 16,384 µg/mL) in comparison to the parent peptide.

***A new method of ionotropic hydrogel formation with extensive control over gelling kinetics*.**

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Hydrogels are an important class of biomaterial. Interest from the biomedical field originates from the ability of hydrogels, derived from both natural or synthetic origins, to inherently or be engineered to mimic physicochemical aspects resembling the extracellular matrix. This has led to many biomedical applications including wound dressings, drug and cell carriers, and as tissue engineering matrices and research continues intensively in these areas.
Polymers forming hydrogels must be stabilised in a continuous aqueous phase using physical and/or chemical crosslinks. This may be achieved via strong covalent crosslinks or physical networks containing transient, reversible junctions based on e.g. ionic and hydrogen bonding or hydrophobic interactions. Of these types, ionic crosslinking offers a convenient route to gelation, without recourse to covalently modify the polymer, and many biocompatible polyelectrolyte systems, such as alginate, pectin, gellan and carrageenan, can be crosslinked under physiological or near physiological conditions. However, ionic gelling kinetics are difficult to control since they are governed by the intrinsic interactions between the ionotropic polymer and the gelling ion and ionic diffusion in water. Attempts to slow down the crosslinking process using retardants, or solid reservoirs of ionic crosslinking agents have been shown to be effective for limited applications. In this presentation control of ionic crosslinking via a novel approach based on tuning the availability of the gelling ions will be described. This approach enables preparation of two stable polymer solutions which upon mixing react to form a crosslinked hydrogel. The time course over which this process occurs can be tuned from a few seconds to tens of minutes, thus representing a highly versatile system. All reactants are contained in the aqueous state and this has particular utility to otherwise challenging applications in cell-compatible 3D structuring with microfluidics and additive manufacturing. Initial demonstrations towards realisation of these approaches will be presented.

Session 8

TBDMS chitosan: properties and utilization as precursor for the synthesis of chitosan derivatives.

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We recently developed an efficient procedure for 3,6-O-di-tert-butyldimethylsilyl (TBDMS) protection of chitosan. TBDMS Chitosan is highly soluble in moderately polar organic solvents, like dichloromethane, and can be used to cast superhydrophic films. We have optimized the conditions for the protection to allow full protection of the hydroxyl groups, in one step, starting from chitosan mesylate. TBDMS can be removed in strong acid or by treatment with fluoride ion. TBDMS-chitosan can be utilized as precursor for *N*-selective modification of chitosan and offers that the advantage that reactions can performed at room temperature with concentrated solutions of the polymer substrate. We have used for the synthesis of 100% dialkyl chitosan and in the synthesis of highly substituted trialkylchitosan, including 100% *N,N,N*-trimethylated chitosan (TMC), which has no trace of O-methylation. TBDMS-chitosan can also be used for quantitative N-acylation of chitosan and thus the degree of substitution can be fully controlled simply by adjusting the equivalent ratio of the reagents. TBDMS chitosan has thus been used for the synthesis of antimicrobial quaternary N-acyl derivatives of chitosan and N-acyl derivatives with covalently linked highly lipophilic photosensitizers, which could be used for cancer therapy. Synthesis with multiple protection groups also been performed by reacting TBDMS chitosan and tertiarybutyloxycarbonyl (boc) protected reagent to obtain guanidinylated chitosan derivatives. TBDMS chitosan is therefore a good starting material for the synthesis of series of chitosan derivatives intended for structure activity investigations.

Poster session

P01

Photocrosslinkable poly(caprolactone diol)/castor oils blends as bioadhesives

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Introduction

Traditional methods of wound closure are suturing and stapling, but are associated with pain, wound infection and low aesthetic results. Therefore, medical tissue adhesives are considered an attractive alternative since, besides wound closure, they can accomplish other tasks, such as haemostasis and the ability of sealing air leakages. UVcurable adhesives offer major advantages, such as fast-curing rate, control of polymerization heat evolution, superior control over the final properties of the material and are ideal for application on weakened tissue.

Methodology

Photocrosslinkable biodegradable bioadhesives based on blends of low molecular weight poly(caprolactone diol) (PCL) and castor oil (CO) were developed. PCL and CO were modified with isocyanate-functional molecules, such as an unsaturated acrylic resin, based on hexamethylene diisocyanate (Laromer® 9000, BASF) or 2-isocyanatoethyl acrylate (IEMA, BASF). Macromers with C=C bonds were obtained which were further crosslinked using Irgacure® 2959 as photoinitiating agent. Flexible, uniform and well defined films were obtained. These were then characterized by swelling capacity evaluation, scanning electronic microscopy, hydrolytic degradation *in vitro*, determination of surface energy and thermal characterization (TGA and DSC). Blood compatibility studies were performed including thrombosis and determination of haemolytic potential. Biocompatibility using human dermal fibroblasts (hFib) and antibacterial activity of the materials were evaluated *in vitro*.

Results

The obtained adhesives presented low values of swelling and a maximum biodegradation value of 20% after 6 weeks in PBS. Surface energies were lower than the ones of skin and blood suggesting that the films will not slough off easily since adhesion forces will be significant. Thermal properties showed that the materials did not suffer any transition at physiological temperature.

Haemocompatibility studies showed that the materials are thrombogenic and that presented no haemolytic effect. Cell culture *in vitro* studies showed that the materials are biocompatible but did not present antibacterial activity *per se*.

P02

Development of hydroxyapatite/modified poly(vinyl alcohol) composites for bone tissue engineering

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Introduction
Significant work has been done investigating interactions of various macromolecules with hydroxyapatite (HAp). However, to the best of our knowledge, there is lack of researches dealing with composites prepared of HAp and poly(vinyl alcohol) (PVA), which has been modified for better drug-loading capacity. In this work, to achieve superior combination of synergic effect and bioactivity of HAp and biodegradability of PVA, composite microgranules were prepared.
Experimental procedure
PVA was modified to introduce -COOH groups by reaction between the -OH groups and succinic anhydride. The microgranules containing HAp/polymer (1:1) were prepared through *in situ* precipitation of HAp in the modified PVA aqueous solutions followed by spray drying of obtained slurries. The microgranules were characterized by Fouriertransform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), scanning electron microscopy (SEM), and differential scanning calorimetry (DSC). The amount of functional groups added to PVA molecules (~15 mol%) was determined by nuclear magnetic resonance spectroscopy (NMR).
Results
FT-IR, XRD and SEM analyses revealed that the spherical microgranules (~2-10 μm) consisted of nanosized HAp crystallites homogenously embedded in the polymer matrix. The presence of -COOH groups was verified by FT-IR. The DSC measurements indicated that decomposition of the composites occurs at lower temperatures than that of pure polymers, respectively. However, the presence of HAp had minor influence on thermal decomposition of the PVA modified with succinic anhydride.
Conclusions
The feasibility of producing microgranules composed of HAp and modified PVA opens the possibility of developing injectable bone fillers or formulation of bone cements with hydraulic setting. The innovative biomaterial could be employed for controlled release of therapeutic agents at wound sites. This preliminary research contributes to further biomedical applications.
Acknowledgement
This work has been supported by the National Research Programme No. 2014.10-4/VPP-3/21 “MultIfunctional Materials and composItes, photonicS and nanotechnology (IMIS^2)”Project No.4 “Nanomaterials and nanotechnologies for medical applications”.

P03
A novel stimuli-responsive polymer-liposome complex for controlled drug delivery
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The development of polymer liposome complexes (PLCs), in particular for biomedical applications, has grown significantly in the last decades. The importance of these studies comes to the emerging need in finding intelligent controlled release systems more predictable, effective and selective, for applications in several areas, such as treatment and/or diagnosis of cancer, neurological, dermatological, ophthalmic and orthopedic diseases, gene therapy, cosmetic treatments and food engineering.
This work reports the development and characterization of an intelligent system for controlled release based on PLCs. The selected hydrophilic polymer was poly(acrylic acid) (PAA) which was synthesized by atom transfer radical polymerization (ATRP) with a cholesterol (CHO) end-group in order to improve the anchoring of the polymer into the liposome. The synthesized polymer was incorporated into liposomes obtained from soybean lecithin and stearylamine, with different stearylamine /phospholipid and polymer/phospholipid ratios (5, 10 and 20% for both cases). The developed PLCs were characterized in terms of particle size, polydispersity, zeta potential, release profiles and encapsulation efficiency. The results showed that the liposomal formulation with 5% of stearylamine and 10% of polymer positively contribute to the stabilization of the complexes. Afterwards, the carboxylic acid groups present in the surface of the liposomes were crosslinked and the same parameters analyzed. The crosslinked complexes showed to be more stable at physiologic conditions and the release profile at different pHs (2-12) revealed that the obtained complexes released all their content at acidic conditions.
In summary, the main accomplishments of this work are: (a) innovative synthesis of CHO-PAA by ATRP, (b) stabilization of the liposomal formulation by incorporation of stearylamine and CHO-PAA, (c) new approach for CHOPAA crosslinking, resulting in PLCs more stable at physiological conditions, and (d) destabilization of CPLs upon slight changes of pH, showing their pH sensitivity.

P04
Atomic layer deposited TiO2 protects highly porous ceramic bone scaffolds from grain boundary corrosion
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Introduction
When highly porous ceramic materials such as bone scaffolds are exposed to corrosive environment, grain boundary (GB) corrosion can have a detrimental impact on the mechanical properties of the scaffold. This study aims to investigate the suitability of atomic layer deposition (ALD) of TiO_2 thin films to protect a macro-porous TiO_2 scaffold from GB corrosion and maintain its mechanical properties in acidic physiological conditions. Two different deposition temperatures were chosen to assess the effect of film structure on the barrier properties of the deposited layers.
Materials and methods
TiO_2 thin films were deposited at 150°C and 250°C on the surface of highly porous TiO_2 scaffolds using chloridewater ALD process. The ALD films were characterised by SEM and AFM imaging, XRD, and ellipsometry. The compressive strength of the ALD coated scaffolds was tested following up to 28 d exposure to 1mM HCl.
Results and discussion
ALD coatings deposited at 150°C were characterised as having amorphous structure, while films deposited at 250°C had anatase crystal structure. The thicknesses of the amorphous and anatase coatings were 26±1 and 22±2 nm, respectively. In addition, strong adhesion between the coating and substrate was observed with no cracking or delamination upon fracture of the scaffold struts. After 7 and 28 days immersion into 1 mM HCl, a significant reduction in the compressive strength of the uncoated scaffolds was observed due to dissolution of siliceous GB phase, whereas both ALD coated scaffolds maintained the initial strength. This indicates that GB corrosion in highly porous scaffolds is prevented under acidic conditions up to an exposure period of 28 days.
Conclusions
GB corrosion in highly porous TiO_2 scaffolds was successfully diminished by atomic layer deposition of both amorphous and anatase TiO_2. The compressive strength of ALD coated scaffolds was maintained in acidic conditions for at least 28 days.

P05
Plasma Treatment in Conjunction with EGM-2 Medium can Enhance Endothelial and Osteogenic Marker Expressions of Bone Marrow MSCs.
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For many tissue engineering applications, an important goal is to create functional tissues *in-vitro*. In order to make such tissues viable, they have to be vascularized. Endothelial cells (ECs) and endothelial progenitor cells (EPC) are promising candidates for restoring vascularization. However, both of them have limited expansion capacity and autologous cells currently do not exist. Therefore, we use bone marrow mesenchymal stem cells (MSC) as a source material for ECs. Growth supplements are commonly used to induce MSC differentiation, and further improvements in differentiation conditions can be made by modifying the cell's growth environment. An example is to pre-treat the culture dish with gas plasma in order to modify the surface functional groups of the material. In this work, we compare the effects of different gas plasmas on the differentiation of MSCs. We treat the dish with different plasma (CO_2, N_2, and O_2) and then induce MSC differentiation with endothelial growth medium-2 (EGM-2). We find that EGM-2 by itself upregulates EC marker CD31 mRNA expression, but not VEGFR2, CD34, or vWF. However, these additional EC marker expressions were increased for cells seeded on plasma-treated substrates. Specifically, we found that N_2 plasma treatment upregulated CD31 and VEGFR-2 mRNA expressions; CO_2 plasma treatment upregulated CD34 and vWF mRNA expressions. The osteogenic markers ALP and osteopontin mRNA expressions were markedly enhanced on all plasma-treated dishes. We also found that plasma treatment in conjunction with EGM-2 can enhance MSCs differentiation into endothelial-like cells and osteogenic-like cells. Our work shows that the effects of the EGM-2 on MSCs differentiation is influenced by the plasma-modified surface chemistry of the substrate. In conclusion, plasma surface modification enhanced EGM-2 effectiveness and induced both endothelial and osteogenic differentiation. Our findings provide a method to enhance EGM-2 based cell differentiation, with consequences for tissue engineering and stem cell biology applications.

P06
Fabrication and characterization of modified titanium dioxide scaffolds for bone tissue engineering
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Tissue engineering scaffolds for replacement of injured and diseased hard tissues such as bones should comprise multitude characteristics including a desirable porosity to ensure vascularisation, desired surface chemistry for deposition of bonelike apatite, ability to support attachment, proliferation, and differentiation of bone cells as well as prevent biofilm formation that might lead to implant failure or strong inflammatory process.

Scaffolds should be threedimensional and highly porous with an interconnected pore network and should supply mechanical support to the skeleton during healing process. Thus, the present study is focused on the preparation and modification of novel tissue engineering scaffolds with suitable mechanical properties and favourable microstructure based on porous TiO_2 ceramic.
Porous TiO_2 scaffolds were produced via polymer foam replica method. Modification of 3D porous TiO_2 scaffolds was accomplished through various methods: vacuum sintering, nanosized TiO_2 coating, polymer or PVA/HAp composite coating. Nanosized TiO_2, polymer and PVA/HAp coatings on TiO_2 scaffolds were obtained via vacuumassisted impregnation of prepared suspensions. The impact of modification on physical characteristics, surface properties, *in vitro* bioactivity and bacteriostasis effect of obtained TiO_2 ceramics was evaluated.
All fabricated scaffolds before and after modification showed interconnected pore network with macropores ranging from 100 to 500 μm. Total porosity of the TiO_2 scaffolds was above 80 %. Infiltration with polymer or PVA/HAp composite slightly reduced porosity of prepared scaffolds, but had a great impact on the mechanical strength. *In vitro* bioactivity testing proved high bioactive properties of the nanosized TiO_2 and HAp/PVA nanocomposite coated TiO_2 scaffolds compared to uncoated. Electroconductive TiO_2 scaffolds have been obtained via sintering under high vacuum conditions. Such materials could enhance bone healing process by providing electrical stimuli in the regeneration site of bone defects.
Acknowledgement: Support for this work was provided by the National Research Programme (IMIS^2) Project No. 4.

P07

Effect of flavonoid-nanocoated implant surfaces on gingival cells and oral bacteria

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Introduction

Many dental implants fail due to the infection and inflammation that walk hand in hand with poor healing and soft tissue integration. Therefore, the development of antibacterial, anti-inflammatory and tissue-regenerative surface modifications constitutes a major challenge to guarantee implant success. In previous studies, we screened among different natural flavonoids and selected quercitrin as a potential biomolecule for periodontal applications. Furthermore, we developed a method to nanocoat titanium surfaces with flavonoids. In this study we aimed at evaluating the antibacterial, anti-inflammatory and soft tissue regenerative properties of quercitrin-nanocoated surfaces.

Methods

Titanium nanocoating consisted in the self-assembly of a quercitrin nanolayer on titanium-hydroxylated surfaces usig an aminosilane as linker agent. First, *Streptococcus mutans* attachment and biofilm formation on the surfaces was analysed. Then, human gingival fibroblasts were used to test cell adhesion. Then, the anti-inflammatory properties and the potential of quercitrin-nanocoated surfaces to boost soft tissue regeneration were also tested under basal and inflammatory conditions. An inflammatory situation was mimicked using interleulin-1-beta.

Results

We found that quercitrin-nanocoated surfaces decreased initial bacterial adhesion while increasing human gingival fibroblasts attachment. Furthermore, quercitrin-nanocoated surfaces increased collagen type I and III mRNA levels and decreased matrix metalloproteinase-1/tissue inhibitor of metalloproteinanse-1 mRNA ratio, which is related to a reduced metalloproteinase-mediated collagen degradation. Quercitrin-nanocoated surfaces also decreased the release of the pro-inflammatory prostaglandin E_2 under basal and inflammatory conditions.

Conclusion

These results suggest that quercitrin-nanocoated surfaces favour gingival cells against oral bacteria, which could enhance soft tissue integration and increase dental implants success.

P08

TiO2-H2O2 as decontamination agent - an in vitro study

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INTRODUCTION: Decontamination of infected dental implants is considered to be a critical step in the successful treatment of peri-implantitis. No standard cleaning protocol has yet been defined, and current treatments often fail the requirement to efficiently remove microbial biofilm and inhibit reinfection. In this study, an *in vitro* assay is developed to dynamically observe biofilm regrowth after decontamination. Oxidative agents were compared, and a potentially more efficient cleaning procedure is presented.

MATERIALS & METHODS: The bioluminescent strain S. epidermidis Xen43 was used to form a 16 h biofilm on titanium coins. The contaminated surfaces were treated with either dH_2O, H_2O_2 or H_2O_2-TiO_2 particles in the presence or absence of near UV-light. After an exposure time of 1 min, samples were placed in fresh media and luminescence was recorded for 16 h as an indicator for viable bacteria. Additionally, SEM images were taken right before and after the treatment.
RESULTS & DISCUSSION: After 16 h incubation, the entire implant surface was covered by a thick, mature biofilm.
Rinsing with dH_2O had no visible effect on the biofilm. Only a few bacteria clusters were observed for H_2O_2 and H_2O_2-TiO_2 but complete decontamination could not be achieved for any of the tested groups. The luminescence assay showed bacteria regrowth for all groups within 16 h after decontamination. The bactericidal effect of H_2O_2 could be enhanced by the addition of TiO_2 particles. Interestingly, this effect was also seen for non-irradiated samples indicating a synergistic effect of TiO_2 not related to its photocatalytic activity. One possible explanation may be the additional generation of radical oxygen species at the metal oxide surface when in contact with H_2O_2.
CONCLUSION: Decontamination with irradiated TiO_2-H_2O_2 could delay bacteria regrowth compared to currently used H_2O_2 treatment and may therefore be used as a cleaning agent for peri-implantitis in the future.

P09

Injectable hydrogel scaffold for bone regeneration: gelation and mechanical properties

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Introduction
Hybrid polyethylene glycol (PEG)-peptide based hydrogels are a versatile platform for bone tissue regeneration. For the application as injectable scaffold a controlled gelation time is crucial. The aim of this study is to assess the effect of pH on gelation kinetics. It is further known that matrix elasticity is a key factor for directing cell fate. The fabricated hydrogels were therefore characterised mechanically on a cellular scale.
Methods
Hydrogels were formed by mixing one of two differently functionalised PEG macromeres with a synthetic peptide at controlled pH. Oscillatory rheometry was used to monitor the gelation kinetics. Elastic moduli were measured by AFM force spectroscopy. Mass swelling ratios were determined gravimetrically. Further, a cytotoxicity study was performed with primary human osteoblasts (hOB).
Results and discussion
Rheometry shows that storage moduli increase slower at lower pH (i.e. faster gelation occurs at higher pH as expected). They reach a plateau at about 10 or 5 kPa determined by the polymer functionalisation. The elastic moduli range from 12.7 to 44.7 kPa depending on polymer functionalisation and increasing with pH. No significant deviation in the mass swelling ratio was detected, indicating equally well-developed networks. The cytotoxicity assay did not show significantly elevated values for any of the gels and time points compared to the control group.
Conclusion
The performed experiments show that the gelation kinetics can effectively be controlled in a clinically relevant time window by altering the pH. Relevant gelation times are reached under acidic and slightly basic conditions for the differently functionalised polymers. Further, a trend of increasing matrix stiffness with increasing pH was shown. None of the investigated systems showed any negative effects on the viability of hOB.

P10

Multifunctional implant design for bone infection treatment

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Introduction: Bone infection is a potentially devastating complication following trauma or surgery.[1,2] Nowadays, 3D scaffolds based on nanocomposite mesoporous glasses with hydroxyapatite nanoparticles embedded in the mesoporous matrix (MGHA) are being widely investigated as promising candidates to use in bone tissue engineering applications and drug delivery systems.[3] Herein hierarchical 3D-multifunctional scaffolds containing levofloxacin, vancomycin and rifampicin for a combined therapy are presented as an alternative treatment of bone infection to the conventional ones.
Methods: 3D scaffolds were fabricated by rapid prototyping technique using a paste formed by aqueous mixture of calcined MGHA powder and Poly-vinyl alcohol (PVA). Previously, both levofloxacin and vancomycin were incorporated into the mesopore structure and polymer matrix, respectively. Finally, 3D scaffolds were coated by a gelatin/glutaraldehyde layer containing rifampicin. *In vitro* release kinetics and microbiological studies with *Staphylococcus aureus* were carried out.

Results: Results show the loading of the drugs (levofloxacin, vancomicin and rifampicin) in different compartments of the scaffolds which display different release profiles for each one depending on parameters such as diffusion coefficient or drug-matrix interactions. The sequential release of different antibiotics provokes a high effectiveness to destroy the bacteria biofilm, which is crucial to eradicate the infection. I*n vitro* preosteoblast culture displays good biocompatibility with complete cell colonization in the entire surface of the scaffolds.
Conclusion: 3D hierarchical scaffolds MGHA-PVA containing different antibiotics with sequential and gradual release kinetics constitute excellent and promising candidates in bone infection treatment with high efficacy to destroy the biofilm. **References**

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P11

Design and preparation of biocompatible zwitterionic Ti6Al4V 3D scaffolds with antimicrobial activity

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Introduction: Large and critic bone defects reconstruction is still a hard challenger in tumor resections, non-unions and some traumatisms.[1] The application of electron beam melting technology (EBM) to implant manufacturing constitutes a promising alternative which provides better biomechanics and customized solutions.[2] Infection is one of the most serious complications of the prosthetic devices, in terms of morbidity, mortality and medical costs.[3] Herein, we report the design and preparation of Ti6Al4V-EBM scaffolds exhibiting zwitterionic surfaces that inhibit both bacterial adhesion and biofilm formation, at the same that allow adequate osteoblast colonization.
Methods: 3D scaffolds were manufactured by EBM (ARCAM B12) using Ti6Al4V-ELI powder (45-70µm). To facilitate the fixation with organic moieties and enhance bioactivity of bare Ti6Al4V surface, they were previously coated with nanocrystalline hydroxyapatite by dip-coating. Subsequently, these 3D pieces were bifunctionalized with organosilanes bearing amine and carboxylate groups to confer a zwitterionic character. The zwitterionic nature was assessed by using Fourier transform infrared spectroscopy and z-potential measurements. Both microbiological assays and cell cultures were also performed.
Results: Results show the effectively of the bifunctionalization process allowing create surface containing -NH_{3+}/COO^{-} zwitterionic pairs homogeneously distributed over the entire surface of 3D Ti6Al4V-EBM scaffolds. The resulting surfaces notably reduce bacterial adhesion and inhibit the formation of bacteria biofilm, while osteoblasts exhibit complete cell colonization.
Conclusion: Surface *zwitterionization* via ceramic coating and biofunctionalization with organosilanes bearing -NH_3 and -COOH groups provides new perspectives for manufacturing Ti6Al4V-based implants for bone tissue repair with antimicrobial properties. **References**

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P12

Cell-responsive biomimetic extracellular matrix material for bone and dental pulp tissue regeneration

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Introduction

Periodontal diseases often lead to bone loss and require surgical debridement. Though traditional therapies are effective in preventing the progression of such diseases, the regeneration of the lost tissues is limited and further therapies are needed. Periodontal tissue engineering that combines supportive biomaterials and stem cells can potentially serve as a promising alternative to conventional periodontal treatments.
Cell adhesion is vital to survival, proliferation and the overall maintenance of tissue integrity. The extracellular matrix (ECM) not only provides anchorage, but also plays a role in determining cell fate and assisting with cellular migration. This project aims to evaluate the cell-responsiveness and regenerative capacity of a novel biomimetic hydrogel material designed to augment periodontal regeneration by controlled local delivery of target-specific biomolecules. **Methods**

A sterile protease sensitive PEG-based hydrogel is synthesized and swollen in mesenchymal stem cell growth media (MSCGM[TRADEMARK]) to form circular discs for *in vitro* testing purposes. Mesenchymal stem cells (P<6) are seeded directly onto the circular hydrogel discs. A preliminary study involves the assessment of cell viability and cell morphology within the gel with varying concentrations of linear and cyclic RGDs, prior to any further customization. Cell migration is to be determined using confocal microscopy and time lapse renders.

Results and Discussion

The biophysical and biochemical properties of the chosen synthetic cell-responsive ECM material influence the homing and behaviour of stem/progenitor cells. The chemical composition, cross-linking density, and enzymatic degradation of the hydrogel defines the capacity of the material to influence cell homing, cell migration, and cell differentiation, and thus the regenerative potential of the cells within the hydrogel material. Cell viability, cell homing and cell fate will be investigated and the effect of targeted and temporally controlled release of IDPs on tissue regeneration will be evaluated both *in vitro* and *in vivo*.

P13

The effect of concentration of calcium phosphate spheres on micro hardness of dentin

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INTRODUCTION

We investigated the effect of calcium phosphate spheres (CPS) on micro hardness of dentin surface during its remineralisation process, which could treat teeth sensitivity.

EXPERIMENTAL METHODS

The tooth slices were applied with CPS-glycerol paste, the concentrations of which were 0.5 wt%, 1wt%, 5wt% and 10 wt%, respectively. After applying, the slices were washed with water and then soaked with PBS solution, which were kept in the oven at 37°C for 7 days.

The measurements were performed by a micro hardness tester with Vickers indenter (Micromet 2100, Buehler, Illinois, US). In the testing, a diamond in the shape of a square-based pyramid is pressed into treated dentin under either 300 or 50 g loads. Further, the reference without paste treatment was tested under 50, 100, 300 and 500 loads. Each dentin surface received 40 indentations, 20 for each load. The duration of load application was 15 s.

RESULTS and DISCUSSIONS

Table 1 shows the average of microhardness decreased as concentration of CPS increased under load 300g as well as 50g. The decrease of average microhardness of dentin could help to confirm the remineralization. We believe the decline of microhardness was caused by new formed layer which is porous.

Table 1 Mean Vickers hardness number (VHN) Values (GPa) and Standard Deviation of dentin at two different indentation loads

Table 2 Mean VHN Values and Standard Deviation of Dentin (reference) at four different loads

CONCLUSION

More remineralization caused lower VHN values of dentin. The VHN values of dentin were, however, affected by variation of indentations loads.

P14

Bone Surface Mimicked Scaffolds for Bone Tissue Engineering

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Altering surface stiffness, chemistry, surface roughness and topography of biomaterials may influence the cellsurface, cell-scaffold interactions. Thus, these factors are important to develop new materials for tissue engineering applications. In this study, bone surface mimicked (BSM) scaffolds were synthesized from biodegradable Poly-L- Lactic acid (PLA) by using Polydimethylsiloxane (PDMS) moulds obtained from bovine femur. Continuously, bone morphogenic protein 2(BMP-2) was loaded into the scaffolds. Subsequently, hydroxyapatite (HA) and collagen (Col I) were immobilized to the BMP-2 loaded and plain PLA scaffolds in order to mimic the natural micro-environment of the bone. In order to characterize the surface properties, Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD) and X-Ray photoelectron spectroscopy (XPS) were performed. According to the SEM results, full coverage of HA and Col1 was obtained over the surfaces. Obtained peaks at 31.7º and 32.8º in X-Ray Diffraction (XRD) and biding energies at 402 and 399 eV X-Ray photoelectron spectroscopy (XPS) analysis for HA and Col I, respectively support the successful chemical modification of the surfaces. Water Contact Angle (CA) measurements were performed in order to understand the wetting behavior

of the prepared surfaces since wettability is one of the majoring effect for cell-surface interaction. As expected, BSM-PLA substrates were found more hydrophobic in compare to the plain PLA due to the increased surface roughness. On the other hand, after immobilization of HA and Col I, BSM-PLA was found more hydrophilic due to the same reason. Additionally, hydrophilic behavior was observed for all kind of substrates modified with HA and Col I where PLA is a naturally hydrophobic material. BMP-2 Loaded Col I and HA Modified Bone Surface Mimicked PLA Scaffolds are expected to promote the osteogenic differentiation of adipose derived stem cells at in-vitro and in-vivo conditions as future studies.

P15

Ex vivo cornea model for studying performance of biomaterial and cell based corneal grafts

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Introduction:
Corneal vision loss is globally the most common reason for permanent visual impairment. Shortage and inadequacy of donor tissue to repair corneal defects has led to a high demand for tissue engineered corneal grafts. Preclinical evaluation of biomaterial and cell based corneal grafts always requires implantation into an animal model. The increasing research on corneal grafts means also an increased need for animal testing. To meet the ethical and economical challenges of animal testing, we have utilized an *ex vivo* cornea model using excised human and porcine corneas for the preliminary testing of biomaterial based corneal grafts.
Methods:
Human corneas unfit for transplantation were obtained from the tissue bank of University of Tampere and excised porcine corneas from a local abattoir. The inner cups of the corneas were filled with agarose-collagen gel and the corneas were cultured partially submerged in culture medium with periodical wetting. Corneal epithelial cells seeded on transparent collagen membranes were implanted to the surface of the corneas. The state of the corneal tissue was determined by fluorescein staining and end-point histological evaluation.
Results:
The *ex vivo* cornea model provided a good platform for testing the biomaterial suitability for corneal implantation and optimization of the implantation technique. The biologically functional cornea model also supported the growth of corneal epithelial cells on the collagen membranes.
Discussion and conclusions:
The *ex vivo* cornea model provides a relatively low cost and simple method for preliminary testing of various biomaterial and cell based constructs for treatment of corneal defects. The corneal model can also serve as a practice platform for implantation technique optimization. The *ex vivo* cornea model has the potential to reduce animal testing in ophthalmological research.

P16

Biocompatibility of decellularized skeletal muscle scaffolds with C2C12 myoblasts

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Decellularization of tissues preserves well stroma as well as original complex microstructure of the extracellular matrix (ECM). Combination of acellular scaffolds with cells represents a great tool for production of new biological constructs in tissue engineering. The tibialis anterior obtained from C57Bl/6 mice was decellularized by osmotic shock followed by SDS extraction and peracetic acid to sterilize the bioscaffold. DNA was removed with DNase. After a thorough washing with PBS buffer the scaffolds were collected for chemical and microscopic characterization or for recellularization. Light microscopy of paraffin-embedded sections proved absence of cell nuclei and cytoplasmic components in decellularized muscle. Transmission electron microscopy of the scaffolds revealed well preserved general microarchitecture including basal laminas and transversely striated collagen fibrils. Immunohistochemical analysis confirmed preservation of proteoglycans and adhesive glycoproteins such as laminins and fibronectin in decellularized scaffolds. The scaffolds were recellularized with C2C12 myoblasts and cultured in vitro for 3, 6, 9 and 12 days in vitro. Histological examination confirmed biocompatibility of the scaffolds as these were successfully reseeded with cells demonstrating the ability of cells to survive, adhere, grow and migrate through this ECM without affecting the scaffold structure. Myoblasts were aligned with the scaffold ECM, they had physiological morphology with a well-established actin cytoskeleton and a centrally located nucleus. We demonstrate preservation of the 3-dimensional microarchitecture and ECM composition in scaffolds obtained after decellularization of the skeletal muscle. The scaffolds are biocompatible, allow cell attachment and preserve a host environment for guiding and spatially organizing cells after recellularization. Decellularized muscle scaffolds can be considered as a promising alternative for construction of muscle tissue replacements to treat incontinent sphincters and extensive post-traumatic and post-surgery muscle ablation. This

work was supported by the Grant Agency of the Czech Republic 15-09161S, Grant of the Charles University in Prague No. SVV-2015-260179 and PRVOUK P37/06.

P17

The effect of different surface modifications of Ti-35.3Nb-7.3Zr-5.7Ta-0.7O alloy on differentiation of adipose tissue-derived stem cells

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Titanium alloys have been extensively applied in orthopedics due to their superior mechanical properties, excellent corrosion resistance and favorable biocompatibility for several decades. Specialized beta titanium alloys are believed to replace the most common Ti-6Al-4V alloy due to their enhanced biocompatibility and reduced elastic modulus. However, the considerable disadvantage of these alloys is relatively low strength (around 550 MPa for TiNb-Zr-Ta alloy). This could be overcome by small oxygen additions. We were able to double the hardness and yield stress of Ti-Nb-Zr-Ta alloy by adding 0.7 wt. % of oxygen.
The next aim of this study was to evaluate the impact of different surface modifications of Ti-Nb-Zr-Ta-O alloy on the proliferation and differentiation potential of cells. Electric discharge machining (EDM) alone or followed by chemical milling and shot-peening was compared to polished surface used as a standard material. Although the polished samples showed the best support for the proliferation of osteoblast-like cells, electric discharge machining treatment promoted superior osteogenic differentiation of adipose tissue-derived stem cells. Quantitative real-time PCR results revealed significantly higher expression of all three evaluated osteogenic markers (alkaline phosphatase, collagen type I, and osteocalcin) in cells cultivated on EDM treated alloys. In addition to expression level, the activity of alkaline phosphatase calculated to cell numbers (analyzed by a Resazurin assay) also confirmed higher values in ASC cultivated on alloys with electric discharge machining surface modification.
This study was supported by the Grant Agency of the Czech Republic (Grant No. 15-05864S), by the Technology
Agency of the Czech Republic (Grant No. TA04011214), and by the Agency for Healthcare Research, Ministry of Health of the Czech Republic (grants 15-33018A and 15-32497A).

P18

Hyaluronan hydrogel/ calcium phosphate composites for medical application

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In the last decade, fabrication and investigation of inorganic-organic composites based on calcium phosphates (CaP) and biocompatible polymers has attracted a lot of interest in the field of bone tissue engineering. These composites have potential application in controlled release drug delivery systems. Hyaluronic acid (HA) is a naturally derived polymer as a major component of the extracellular matrix, mediates various cellular activities, and has been widely used for tissue engineering scaffolds. The combination of CaP compounds and HA can provide additional advantages compared to both materials individually.
HA/CaP composite possesses the fundamental characteristics of biomaterials such as bioactivity, biomechanical similarity to the bone tissue, processability, and biodegradability. In the current research calcium phosphate and hyaluronan hydrogel composites with high inorganic particle load were prepared and characterized.
Calcium phosphates were obtained via wet precipitation reaction from calcium oxide and orthophosphoric acid. Solutions with four different HA concentrations were mixed with CaP nanoparticles and crosslinked using 1.4butanediol diglycidyl ether (BDDE). Seven different compositions were prepared - with mass fraction (wt%) of HA 30 wt% - 90 wt%. Obtained samples were freeze-dried and characterized using Fourier transform infrared spectroscopy and X-ray diffraction. Swelling behavior of the composites was studied by gravimetric method. BDDE crosslinked HA/CaP hybrid hydrogels were successfully fabricated with inorganic phase mass fraction from 10 wt% - 70 wt%. During the swelling experiments it was found that pure HA samples tend to disintegrate with time in PBS, but composites containing CaP nanoparticles maintained their integrity. Furthermore, the amount of CaP influenced swelling behavior. These findings allow the optimization of hybrid hydrogel to meet the needs of various tissue engineering applications.

P19

Human Pluripotent Stem Cell -Derived Neural Cultures in Bioamine Crosslinked Gellan Gum Hydrogels

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Introduction:
Extra cellular matrix (ECM) -mimicking biomaterials can be utilized both in 3D tissue model and cell craft development. In neural field hydrogels are most prominent due their similar physical properties compared to native brain ECM. In addition to physical support the optimal biomaterial also offers attachment sites to cells. Due to divergence between species it is important to study human derived cells when the ultimate goal is clinically relevant application.
Aims:
The aim of this work was to study the suitability of gellan gum based hydrogels as culturing matrix for human neural cells and 3D neural network formation.
Methods:
Gellan gum (Gelzan®, Sigma-Aldrich, Finland) was gelated using bioamine as crosslinking agent. Gellan gum hydrogels were functionalized by adding ECM derived biomolecules as physical mixture. Human pluripotent stem cell -derived neural cells were cultured under the hydrogels, on top of the hydrogels and/or inside the hydrogels. Laminin protein coating was used as positive 2D growth control and non-coated cell culture plastic as negative control. Cultures were monitored during experiments and at end point, characterized using viability and immunocytochemical analysis.
Results:
Hydrogels crosslinked using a bioamines were non-cytotoxic. Cultures showed neuronal maturation either under or on top of the hydrogels (2D cultures) and mixed inside the hydrogels (3D cultures). Addition of ECM derived molecules to the hydrogel enhanced neural cell migration.
Conclusions:
According to our *in vitro* studies the gellan gum was non-harmful and promising growth matrix but needs functionalization in order to obtain cell migration.

P20

Sol-gel coatings to prevent periimplantitis

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In spite of the great results obtained in previous works in the osseointegration of titanium implants modified with hybrid sol-gel coatings, there is another problem in implantology that should be overcome: periimplantitis. This has been defined as an inflammatory reaction associated with loss of supporting bone around the dental implant, caused by oral bacteria.
It is clear the need of finding new systems that prevent periimplantitis in various moments after the implantation. Thus, the main aim of this work is the development and the evaluation of hybrid silica sol-gel coatings doped with several antimicrobial agents.
Different coatings were synthesized by the acid catalysis sol-gel method from the combination of some alkoxysilane precursors: methyltrimethoxysilane (MTMOS), 3-glycidoxypropyltrimethoxysilane (GPTMS) and tetraethyl orthosilicate (TEOS). These formulations were functionalized with bioactive molecules (chlorhexidine, octenidine...) during the synthesis process. The chemical characterization was carried out by Infrared Spectrometry and Nuclear Magnetic Resonance, the morphological one by cross-cut test and the biological evaluation was done with *in vitro* assays. Other tests were performed to complete the characterization: determination of hydrophobicity/hydrophilicity, hydrolytic degradation test and silicon release test. Finally, the concentration of released drug was measured spectrophotometrically.
The successful obtaining of transparent, homogeneous and uniform coatings where antimicrobial agents are effectively immobilized can be confirmed from the chemical and morphological characterization. Furthermore, the bioactive molecules incorporation was made without altering the material chemistry. Moreover, *in vitro* studies showed the biocompatible, bactericide and non-cytotoxic behaviour of these materials.
The results of this study confirmed that the new hybrid organic-inorganic silica sol-gel coatings are a great contribution to the dental implantology. The coatings developed in this work were biodegradable, biocompatible and osteoinductors. Finally, they provide an environment with antibacterial capacity to prevent and eliminate the periimplantitis. *In vivo* studies are being carried out and results will be discussed further.

P21

Influence of carboxylic acid functionalized graphene on polysulfone porous films performances

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Introduction
Amongst such wide variety of biomaterials, polysulfone (PSF) found its usefulness as a result of its high chemical and thermal stability, resistance against most sterilization methods and biocompatibility. However, its hydrophobic character is often not desired for certain applications, therefore different strategies are applied to overcome such diasdvantage. This study reports the investigation of carboxyl graphene (G-COOH) potential in optimizing PSF porous films. PSF films with 0.25 to 1 % by weight G-COOH were prepared through wet-phase inversion technique and characterized by Raman spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), profilometry, contact angle (CA), dynamic mechanical analysis (DMA), tensile tests, as well as biocompatibility and cells cytoskeleton monitoring.
Results
Raman spectrometry, XRD and TEM revealed the well-dispersed G-COOH layers within the PSF matrix, leading to the formation of homogenous structuration. Larger pores formed in the presence of G-COOH were evidenced by SEM, as well as changes in selective layer thickness and macrovoids shape. A ~50 % reduction of surface roughness was computed through profilometry. Interestingly, G-COOH rich areas on PSF porous films were favourable for cells cytoskeleton development without bringing further cytotoxic potential. **Conclusions**
PSF / G-COOH composite porous films were prepared by wet-phase inversion method and exhaustively investigated in terms of structure, morphology, topography, hydrophilic-hydrophobic nature, thermal and mechanical performances and biocompatibility. Results highlighted the importance of G-COOH concentrations in optimizing materials morphological and topographical features, as well as stress transfer within composite materials. The addition of G-COOH favored cell proliferation and attachment to PSF / G-COOH composites. Such results could meet several requirements for biomedical applications such as hemodialysis and tissue engineering applications.
Acknowledgements
This work was financially supported by The Romanian National Authority for Scientific Research, Executive Agency for Higher Education, Research, Development and Innovation; Project Number PN-II-TE- 17/2013 and PN-II-PCCA14/2012.

P22
Hybrid Sol-gel Silica/gelatin Fibres via Solution Blow Spinning for Tissue Regeneration
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Traditional bioactive glass based materials have been shown to stimulate bone regrowth but exhibit brittle behaviour. Hybrid materials, constituting an organic and an inorganic phase, combine the beneficial properties of bioactive glass with the flexibility of organic polymers. Solution blow spinning (SBS) is a method for producing micro- and nanofibers at high rates by injecting polymer solutions into a stream of air blown from an outer concentric nozzle. Spinning largely aqueous solutions is challenging; typically volatile organic solvents are used to aid rapid fibre formation.
In this work aqueous formulations of tetraethyl orthosilicate(TEOS)/gelatin are developed and optimised for hybrid fibre production via SBS. TEOS is first hydrolysed (typically 5.7 ml TEOS, 1.2 ethanol, 0.5ml H_2O and 0.45ml of 1M HCl) and then mixed with 5.7 ml of 10 wt.% aqueous gelatin solution. The solution is then heated to 60 °C and the solvent partially evaporated. The time dependent viscosity (due to evaporation and polycondensation) is monitored until the solution is suitable for spinning. A syringe driver injected the precursor at 200 $\mu L \cdot min^{-1}$ and SBS was conducted using air at 30 psi, with a working distance of ~20 cm to evaporate the remaining solvent and form fibres. Dry, cool air was supplied via a water- and oil-free compressor. The fibres were characterised by scanning electron microscopy and their inorganic content verified by thermogravimetric analysis.
The pH (range 3.5-4) and mixing temperature affected the rate of polycondensation. The SBS head was modified to incorporate an inline heating element to deliver a controlled stream of warm air (>25 °C) to force evaporate the solvent, aiding fibre formation. Fibres of roughened morphology and diameters 800 nm - 5 µm were successfully spun from solutions within the viscosity range (52-157 mPa·s).

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P23

Human adipose stem cells cultured on braided polylactide scaffolds is a potential approach for tendon tissue engineering

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Our aim was to find an efficient strategy to produce *in vitro* a potential tendon tissue engineering construct. The preliminary tests included the optimization of the tenogenic differentiation medium (TM) for the human adipose stem cells (hASCs), and the screening of biomaterials and tissue engineering scaffold structures. The optimized TM enhanced significantly the tenogenesis of the hASCs. The braided poly-L-D-lactide 96L/4D (PLA) filament scaffolds in the TM condition supported hASC viability, proliferation and tenogenic differentiation compared to the foamed poly(L-lactic-co-ε-caprolactone) 70L/30CL (PLCL) scaffolds. The braided PLA 96/4 scaffolds supported the formation of a uniform layer of the hASCs when cultured in the TM condition compared to the maintenance medium (MM) condition after 2 weeks of culture. The total collagen content and the gene expression of tenogenic markers of the hASCs was significantly higher in the TM condition after 14 days of culture. The elastic modulus of the braided PLA 96/4 scaffold resembled more Young's modulus reported for native Achilles tendon. Our study showed that the optimized TM is needed for the efficient and rapid *in vitro* tendon-like matrix production of the hASCs. The braided PLA 96/4 scaffolds combined with TM significantly enhanced tenogenic differentiation of the hASCs. The proposed tendon tissue engineering applications represent a novel and feasible application with wide applicability.

P24

Synthesis of four-arm poly(ethylene glycol)-nitrophenyl carbonate for PEG-peptide hydrogels

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In order to better simulate extracellular matrix properties, poly(ethylene glycol) (PEG)"'peptide hydrogels with an unprecedented degree of control over stiffness, degradability and cell adhesion ligand presentation are under development. These highly modular and orthogonal scaffolds will enable encapsulation of stem cells, for analysis of individual or combined effects of matrix properties on differentiation.
The present research concerns synthesis of PEG4NPC (four-arm PEG-nitrophenyl carbonate), which can be crosslinked via amino groups within peptides, such as those on N-terminal residues or the side-chain of lysine.
The reaction was performed under anhydrous conditions in a nitrogen chamber:
PEG4OH (four-arm PEG-hydroxyl) dissolved in 10 mL anhydrous dichloromethane (DCM)
4-nitrophenyl chloroformate (4-NPC) dissolved in 15 mL DCM at 3 M equivalence per OH group
PEG4OH solution added dropwise to 4-NPC solution and stirred at room temperature for 72 h
DCM evaporated overnight, step 5 conducted three times
Product dissolved in 3 mL DCM, precipitated under centrifugation using 180 mL diethyl ether (DE) for 20 min at 4 °C, supernatant aspirated
Solvent evaporated overnight, product dried at 70 °C under vacuum for 1 h before lyophilisation overnight
The level of NPC coupling was determined by quantifying 4-NPC dissociation in 1 M NaOH by measuring absorbance at 405 nm and using the extinction coefficient of 18,000 $M^{-1} \cdot cm^{-1}$, similar to measurement of 4nitrophenyl phosphate in the alkaline phosphatase assay. 93% coupling was achieved.
This facile protocol may be used to synthesise PEG4NPC, which can then be cross-linked using peptides containing various introduced functionalities, such as adhesiveness and degradability.
Acknowledgements: The authors wish to acknowledge Oommen Varghese for advice and Jane & Aatos Erkko Foundation, The Company of Biologists, Journal of Cell Science and Royal Society of Chemistry for funding.

P25

Investigation of ionic strength on aqueous TiO2 slurries and microstructure of sintered TiO2 bone scaffolds

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INTRODUCTION

Highly porous and interconnected ceramic titanium dioxide (TiO_2) foams have been identified as promising scaffold materials for restoration of bone defects. The objective of this study was to investigate the effect of different cations on aqueous anatase slurries and the influence of altered slurry behaviour on properties of sintered TiO_2 bone scaffolds.

EXPERIMENTAL METHODS

The influence of different cations (Na^+, K^+, Ca^{2+}, Mg^{2+} and Sr^{2+}) on the dispersion properties of TiO_2 slurries and the microstructure of porous TiO_2 scaffolds was investigate. Chloride salts of these cations were added into acidic (pH = 1.5 - 1.7) aqueous anatase dispersions and scaffolds were prepared from the dispersions with foam replication method. Zeta potential and hydrodynamic diameter of dispersed TiO_2 particles and viscosity of the prepared slurries were measured. 3D pore architecture of sintered scaffolds was analysed using microCT, while SEM imaging with EDX was used to investigate the microstructure of TiO_2 scaffolds. In addition, compressive strength of TiO_2 scaffolds was measured.

RESULTS AND DISCUSSION

Regardless of the cation species and concentration, increased ionic strength resulted in reduced zeta potential, and thereby, increased slurry viscosity, increased strut size, and improved scaffold strength without reduction in porosity. Divalent cations had stronger influence on the slurry behaviour than monovalent cations and resulted in considerably coarser grain size. Furthermore, Sr^{2+} and Ca^{2+} ions at 0.1 M concentration resulted in the formation of a prominent grain boundary (GB) phase, which in the case of Sr^{2+} addition appeared to alter fracture mechanism of the scaffold struts.

CONCLUSION

Divalent cations in the slurry resulted in large grain size, formation of a prominent GB phase, and improved compressive strength of TiO_2 scaffolds. Further investigation on influence of the development and dissolution of GB phases in scaffolds with high concentration of divalent cations is required.

P26

Synthesis of Gelatine-Methacrylate Hydrogels as Cell Carriers

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Introduction: Selection of scaffold material is of crucial importance in tissue engineering applications in terms of cell behavior and function. The selected material should have the desired properties for the intended use including but not limited to biocompatibility, mechanical and morphological structure features such as strength and porosity, and in most cases, biodegradability. Among many other options, hydrogels are one of the preferred matrices that provide efficient gas and nutrient exchange. In this study, we aimed to optimize the synthesis parameters for gelatinemethacrylate (GelMA) preparation and cell encapsulation.**Material:** Photo-crosslinkable gelatine-methacrylate (GelMA) synthesis was started from the preparation of methacrylated gelatine from a gelatine solution (8 % w/v) that was reacted with different concentrations of methacrylic anhydride to yield GelMA prepolymer. The prepolymer solution was then prepared by mixing 5% and 7% (w/v) GelMA powder and 0.5% (w/v) photoinitiator in phosphate buffered saline at 70˚C to obtain a homogeneous mixture. Cell encapsulation was formed by suspending cell (MG63) pellet in GelMA prepolymer solution followed by photocrosslinking by UV light. Cell viability was determined by live/dead assay kit.**Results:** GelMA prepolymer prepared by using 8 ml of methacrylic anhydrade was found suitable for preparation of the hydrogels since other (i.e. 4 or 16 ml) amounts failed to give stable gels. The structure and swelling behaviour of hydrogels were analysed by using different techniques. According to live/dead assay results, cell viability was confirmed following 21 days.**Conclusion:** We demonstrated the use of GelMA hydrogels for cell-biomaterial applications with this preliminary study. According to results of swelling and cell viability analysis, the use of 7% GelMA was found appropriate. The results affirm that an increased concentration in GelMA increased cellular attachment and cell encapsulation ability. GelMA hydrogels are suitable for mm-sized cell culture matrix.

P27

Morphological and radiological evaluation of bone in animals with experimental osteoporosis after implantation of HAP/TCP bioceramic granules

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Introduction

Osteoporosis is a systemic skeletal disease characterized by low bone mass that may lead to weak and fragile bones. Within this study Calcium phosphate (CP) bioceramics and new methods for enhancement of bone volume and mechanical properties - local treatment of an osteoporotic bone are investigated.
Materials and methods
Experimental osteoporosis was initiated in 20 rabbits. On proximal end of the femur 4 mm hole was created and filled with a biphasic CP bioceramic granules 1.0-1.4 mm synthesized by aqueous precipitation technique (10 rabbits). The control group consisted of 10 rabbits with the analogous defect of osteoporotic bone without implantation of bioceramic granules. After 3 months animals were euthanased. The established osteoporosis and effect of implantation was evaluated by radiological and morphological methods. Tissue samples were stained according the standart immunohistochemical methods and expression of osteocalcin (OC) and osteopontin (OP) was examined. Bone microarchitecture and optical density was evaluated with micro-computed tomography using InSyTe FLECT/CT system. The field of the measured bone density was 5 mm^2, the obtained measurements were registered in Hounsfield unites (HU). Results
Histological samples from control group showed local bone atrophy with decresed size of bone trabeculae prooving development of osteoporosis. In experimental group increase of osteocalcin expression was observed. Also osteopontin what is active in bone remodeling was found increased in all samples of experimental group, but less obviously as osteocalcin. Bone optical density was decreased in control group (mean 1497 HU), reaching 3510 HU in experimental group. Conclusion
Biphasic calcium phosphate bioceramic granules implanted in osteoporotic bone showed local bone regeneration and remodeling potential by increase of osteocalcin, osteopontin and bone optical density.
Acknowledgement
This work has been supported by the National Research Programme No. 2014.10-4/VPP-3/21 “MultIfunctional Materials and composItes, photonicS and nanotechnology (IMIS^2)”Project No. 4 “Nanomaterials and nanotechnologies for medical applications”.

P28

A comparison of the mechanical properties of commercial and cutting-edge experimental calcium phosphate cements

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Introduction

Calcium phosphate cements (CPCs) have been widely studied for bone repair. There are two main types of CPCs, brushite and apatite. In this study, the mechanical properties of particularly promising experimental brushite and apatite cements were evaluated in comparison to commercial brushite- and apatite-based cements (chronOS™ Inject and Norian® SRS®, respectively).

Methods

Compressive, diametral tensile and biaxial flexural strength in wet (or moist) and dry conditions were assessed for two experimental cements, a brushite cement[1] and an apatite cement[2] and two commercial cements (chronOS™ Inject and Norian® SRS®), as well as cement porosity, composition and microstructure.

Results

The experimental brushite cement had the highest compressive strength in both wet and dry conditions (57.2 ± 6.5 MPa before drying and 69.5 ± 6.0 MPa after drying). This cement also presented the highest diametral tensile strength (10.0 ± 0.8 MPa) and biaxial flexural strength (30.7 ± 1.8 MPa) in wet condition. It was also the cement that showed the lowest porosity (approx. 12%).

Discussion and conclusions

The novel experimental cements were found to show better mechanical properties than the commercial cements, in all loading scenarios. The effect of water content was found to depend on cement type, with some cements presenting higher mechanical properties after drying and some no significant difference after drying. This is the first time that the diametral tensile and biaxial flexural strength in both wet and dry conditions are presented.

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P29

The design of calcium phosphate particle for tooth hard tissue remineralization

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Dental caries is a world-wide oral disease. At the initial stage of caries lesions, cariogenic bacteria attack tooth enamel, leading to demineralized areas on the tooth surface. Therefore the investigation includes the design of calcium phosphate (CaP) particles with caries preventive effect due to bioavailable calcium and tailored particle morphology. The aim is to obtain CaP particles with properties close to enamel crystals (20-100 nm) and dentine tubules (2-4 μm). CaP synthesis was carried out by wet precipitation method at 45°C. CaO and H_3PO_4 were used as precursors. The pH of suspension was adjusted to ensure the Ca/P ratio under 1.67 to obtain calcium deficient hydroxyapatite. After the synthesis precipitates were aged at room temperature for 16 hours. One part of the product was spray-dried (spCaP) and remaining part - left as paste (pCaP). The evaluation of chemical and phase composition was done by X-ray diffraction (XRD) and Fourier transform infrared spectrometry (FTIR). For examination of particle morphology scanning electron microscopy (SEM) was used. FTIR analysis showed a characteristic spectra of apatite and the presence of HPO_4^{2-} group was observed at 879 cm^{-1}. XRD patterns after synthesis confirmed apatite phase with low crystallinity, while sintered samples had sharper diffraction maximums, indicating that crystallinity has increased. The Ca/P ratio was in the range from 1.64 to 1.60. SEM micrographs showed nanorods (length 50-200 nm, diameter 25-60 nm) for pCaP and spherical agglomerates (1-10 μm) for spCaP samples. The obtained CaP are chemically very similar to dental hard tissues. In addition, the morphology of pCaP particles is compatible with enamel crystals, while size of spCaP agglomerates fits well with the dimensions of dentine tubules. The combination of pCaP and spCaP have a potential to decrease a risk of caries development and this hypothesis will be tested in *in vitro* studies.

P30

Biomimetic calcium phosphate nanoparticles with variable crystallinity degree for bone tissue engineering

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Biomimetic features of modern biomaterials are important when designing new materials for tissue engineering. When it comes to development of new bone engineering material most of the material scientists mimic the composition (phase, chemistry), appearance (shape, size) and biological behaviour (biocompatibility, bioactivity) of both particles and monoliths for the specified application. Bone is composed of calcium phosphate (CaP) nanoparticles that resembles composition of ion-substituted hydroxyapatite with low crystallinity. Crystallinity of inorganic biomaterials is usually not considered a top priority property when designing new tissue engineering system.
Our work is aimed on development of biomimetic CaP nanoparticles with variable crystallinity degree to suit patient specific needs. We have synthesized amorphous and partially crystalline CaPs with a novel technology. Technology allows obtaining amorphous CaP nanoparticles with high specific surface area (> 130 m^2/g) using conventional hot air drying. Study is devoted to comparison of drying methods (air or freeze drying) and high temperature crystallization studies of amorphous and low crystalline CaPs.
Materials were tested with state of art set of methods: Fourier transform infrared spectrometry, powder x-ray diffraction, BET, scanning electron microscopy with EDS, differential scanning calorimetry and thermal gravimetry, high temperature microscopy.
Results showed transformation of amorphous powders to beta tricalcium phosphate and hydroxyapatite phases upon heating with onset of the process over 600-650°C. Hot air drying produced both amorphous and partially crystalline CaPs, but freeze-drying produced amorphous CaPs. Crystallinity of the final product was influenced by synthesis pH in case of air-drying. Study allowed us to prepare biomimetic and stable (for at least 8 months) CaP nanoparticles with variable crystallinity degree for bone tissue engineering needs.
Acknowledgements: National Research Programme No. 2014.10-4/VPP-3/21 “MultIfunctional Materials and composItes, photonicS and nanotechnology (IMIS^2)”Project No. 4 “Nanomaterials and nanotechnologies for medical applications”and COST Action MP1301.

P31

Alginate microbeads - strategies for stabilising alginate gels with intermediate G content

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The use of alginate microbeads as an immunoisolation system for the entrapment of pancreatic islets has shown great potential throughout the years. However, destabilisation of the gel network under physiological conditions is one of the limitations of the technology. Alginate is a binary heteropolymer containing 1,4-linked β-**D**-mannuronic acid (M) and α-**L**-guluronic acid (G) residues, known for its gel forming properties in the presence of divalent cations. Alginates with a high content of G (>60%) produce mechanically stable beads with calcium and barium ions, whereas alginates with a lower G content (40%) have been shown to form mechanically unstable beads with calcium and barium ions. It has been argued that intermediate to low G content in the alginate may be

beneficial in relation to cellular overgrowth on alginate beads, as well as to the viability and function of encapsulated cells. In the present study, the stability of alginate beads with intermediate G content (46% G, from *Laminaria hyperborea* leaf) was systematically investigated with respect to size and polymer distribution by consecutive saline treatments and confocal laser scanning microscopy, and was ultimately compared to the widely studied and mechanically stable high G alginate from *L. hyperborea* stipe. High-G alginate beads exerted higher stability with respect to both size and polymer distribution compared to intermediate-G alginate beads. However, the size stability of alginate beads with intermediate G content was improved through the use of barium as cross-linking ion instead of calcium. Additionally, the initial inhomogeneous polymer distribution of the alginate beads with intermediate G content was unstable in solutions containing non-gelling ions, yet the inhomogeneity profile was partly conserved by the inclusion of physiological amounts of calcium in the washing and storage solutions. Lastly, addition of free G-blocks to alginate bead formulations may provide a stabilising effect on the inhomogeneity profile.

P32

Collagen-Carboxylmethyl Cellulose-Tri Calcium Phosphate Multi-Lamellar Cryogels for Tissue Engineering Applications

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In order to regenerate or repair a tissue due to trauma or congenital defects; researchers may use biomaterials as proper scaffolds that support cell growth and to allow new tissue formation. In this study, collagen-carboxymethyl cellulose-tricalcium phosphate cryogels were prepared for diverse biomedical applications. Further chemical and structural characterization were performed by Fourier transform infrared spectra (FTIR), thermogravimetric analysis (TGA), X-ray crystallography (X-RD) and scanning electron microscopy (SEM). Mechanical properties were tested by unconfined compression test. Moreover, hemocompatibility of the cryogels were also evaluated by basic biochemical blood testing. Chemical and structural analysis results demonstrate the well achievement of the crosslinking without any major alteration in collagen and carboxymethyl cellulose with a thermally and structurally stable blend formation. Scanning Electron Micrographs demonstrate the multi-lamellar formation with macro and micro pore composition which can correlate with water uptake results of the cryogels. Hemocompatibility evaluations exhibited that the cryogels are non-toxic and blood-compatible. The overall results including mechanical testing these TCP consisting collagen/carboxylmetylcellulose cryogels may have potential use as a material for hard tissue regeneration.

P33

Design of experiments approach for structural optimization of antibacterial and hemolytic properties of chitosan derivatives

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Experimental Design approach was successfully used to guide the synthesis and determine the structure-activity relationship for antimicrobial derivatives of the biopolymer chitosan. Specialized software with D-optimal design capabilities was used to create a library of chitosan derivatives with optimal structural variation in order to conduct a detailed investigation of the structure-activity relationship. The derivatives contain three substituents: *N*,*N*,*N*trimethylamine, *N*-acetyl and *N*-stearoyl at different degrees of substitution (DS) on the 2-amino group of chitosan. The design matrix consisted of 14 target materials that were synthesized in 'one-pot synthesis' using TBDMSchitosan as the precursor to allow precise control of the DS. The antibacterial activity (MIC) towards the Gram positive bacteria *Staphylococcus aureus* and the Gram negative bacteria *Escherichia coli*, hemolytic activity (HC_{50}) towards human red blood cells and solubility of the chitosan derivatives were used as the responses in the model. The response surface model was refined by removing the interaction terms to improve the statistical significance and predictive power of the model. The model showed that an optimum combination of factors resulted in high activity against *Staphylococcus aureus*, whereas a similar and more linear relationship between the factors was observed for *Escherichia coli*, hemolytic activity and solubility of the chitosan derivatives.

P34

One-pot synthesis of cooligopeptides and peptide functionalized aliphatic polyesters prepared by chemoenzymatic synthesis

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The need for new materials within biomedical applications continues to increase and so does also the demand for new designs of materials and optimizations for their properties. Aliphatic polyesters (which are biodegradable and biocompatible) have over the past decades been used a lot within this field.[1] However, their performance in regenerative medicine is limited by the fact that they are not bioactive and therefore cells cannot interact or attach very well to the material.[2] To address these complications we have synthesized random and block cooligopeptides of lysine and alanine by chemo-enzymatic synthesis using Papain (*Caracia papaya*) as catalyst. In a second step we then used the same method to design bioactive copolymers, where an aliphatic polyester was functionalized with oligopeptides through chemo-enzymatic synthesis. This is a promising synthesis technique since it is environmentally friendly, cost effective and gives high purity peptides.
The synthesis method has been described in our previous work.[3] Alanine and lysine ethyl esters were dissolved together with the catalyst in a phosphate buffer solution (PBS) (40ºC) and analyzed after 30 min. The aliphatic polyester was synthesized according to research by T. Fuoco et al. through ring opening polymerization using a stannous octoate as catalyst.[4] The cooligopeptides and functionalized polyester were characterized by proton and carbon-13 nuclear magnetic resonance (^{1}H-NMR, ^{13}C-NMR) and by Size exclusion chromatography (SEC). The cytotoxicity was analyzed by a standard cell viability test.
From the ^{1}H-NMR and ^{13}C-NMR results it was shown that the cooligopeptides and peptide functionalized polyesters were successfully obtained. The cell viability test also showed that the materials were not cytotoxic. From these results it can be confirmed that chemo-enzymatic synthesis is an effective and simple synthesis method not only for designing different peptides and but also for functionalization and bioactivation of polyesters.

P35

Analysis of diffusion of hydrogel contact lenses and numerical modelling

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Hydrogel (or 'soft') lenses have had a huge impact on the contact lens market since they were first introduced in the late 1960s. These lenses are termed “soft”since they are made from water-swollen, cross-linked, hydrophilic polymers. Several classes of macromolecular systems proved to be particularly suitable for the contact lenses applications: the major ones are the glycol methacrylates, above all, poly(2-hydroxyethyl methacrylate) (pHEMA) and its copolymers with methacrylic acid, the classic based on 1-vinyl-2-pyrrolidone (VP) and its copolymers with HEMA and alkyl methacrylates. In addition to these monomers and their copolymers is poly(vinyl alcohol) and its derivatives for contact lens systems. These polymeric systems are expected not only to improve the water content of the contact lenses but the permeability to oxygen, which are crucial properties.
The commercial soft contact lenses used in this study are Etaficon A (1-Day acuvue moist, Johnson & Johnson Vision Care (Limerick, Ireland)). Diclofenac sodium has been selected as a model drug. In this study, we examined various conditions of drug release and drug diffusion experiments, such as different concentrations of drug solutions (5 mg/ml, 1 mg/ml, 0.5 mg/ml and 0.2 mg/ml) and different temperatures (25°C and 35°C) throughout soft contact lenses. Diclofenac analysis was carried out by HPLC.
It was found that the concentration of drug and temperature affects the drug uptake in contact lenses and release from lenses. It is noted that diffusion increase with rise in temperature for each concentration solution. Quantity of diffusant independent of temperature at the end of experiment, diffusion rate is changed only.
The numerical model constructed for this study. The model has a good agreement with experimental data and can be used as a design tool for the development such ophthalmic delivery system as contact lenses.

P36

Nano-Conjugates based on Chitosan for Photochemical Internalization Based Cancer Therapy.

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Photochemical internalization (PCI) is a novel technology, which utilizes selected photosensitizers (PS) in combination with light excitation, to induce release of endocytosed hydrophilic drugs so they can reach their target before being degraded in lyzosomes. This therapy has been shown to be effective in the clinic but the efficiency of could potentially be further improved with polymeric nanocarriers. These would allow for tumor selective accumulation due to the enhanced permeation and retention (EPR) effect. The aim of the study was to develop synthesize and investigate nanoconjugates, that composed of the highly lipophilic PS, TPP and TPC, covalently linked to carriers based on chitosan. TBDMS protected chitosan was utilized for efficient synthesis of highly substituted nanoconjugates. The structure was determined by 1-H and solid state NMR. The physiochemical characteristics were also investigated and the PCI effect in vitro and in vivo. Fluorescence, NMR

and dynamic light scattering investigations showed that the nano-conjugates formed nanoparticle like structures with average size of nanoparticles was in the range 100-300 nm. The nanoconjugates were effective for PCI mediated gene delivery in human colon carcinoma cell line. TPP nanonconjugates were effective for PCI based gene delivery. Preliminary in vivo experiments showed that TPC conjugates could be used to treat tumor bearing Hsd:Athymic nude-Foxn1nu female mice. These results showed chitosan based nanoconjugates, induced a strong PCI effect are therefore promising for PCI based cancer therapy.