Nanotechnology in Regenerative Medicine: What Have We Learned and Where are We Going ?

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INTRODUCTION: Although medical devices are extensively used to treat damaged tissues, many do not last for the lifetime of the patient. For example, for percutaneous metallic devices, poor skin growth around these areas fails to protect against bacterial invasion, thus, leading to infection at the skin interface and even into the bone. Nanotechnology, or the use of materials with at least one dimension less than 100 nm, may provide the answer. Previous studies have suggested that a surface nanotopography promotes the growth of numerous cells, in particular osteoblasts. In this study, results of improved keratinocyte and osteoblast functions on anodized nanotubular and nanorough titanium surfaces that would be useful to improve skin sealing and bone bonding on metals for external fixation devices are presented.

METHODS: *Surface Preparation*: Surface modification techniques used here to create anodized nanotubes and nanorough features on titanium include anodization and electron beam evaporation. Anodization (an electrolytic passivation process used to increase the thickness of the natural oxide layer on metal surfaces) was used to create nanotubes while electron beam evaporation (high electron beam bombardment used to deposit a material, in this case titanium, onto another material, also titanium here) was used to create nanotextured features.

Cyto- and Biocompatibility Assays: To quantify the influence of these titanium nanotubes and nanorough features on human keratinocyte (CRL-2309 ATCC) and osteoblast (CRL-1213 ATCC) functions, cell seeded titanium plates were incubated at 37°C with 5% CO_2/95% air for 4, 24, 48, and 72 hours. After this time period, substrates were rinsed with phosphate buffered saline, fixed with formalin based acetate buffer, stained with DAPI dilactate, and counted under fluorescence microscopy. In vivo studies were also conducting by implanting various titanium abutments into resected rat femurs for up to 6 months. All experiments were run in triplicate and repeated three times.

RESULTS: The anodized titanium surfaces clearly possessed nanotubes approximately 80 nm in length (Fig. 1). The electron beam evaporated titanium surfaces possessed an increased nanometer surface roughness compared to their counterparts the unanodized, conventional titanium (Fig. 1). Results of this study provided the first evidence that skin and bone growth on the anodized nanotubular and nanotextured titanium was significantly greater compared to the conventional titanium

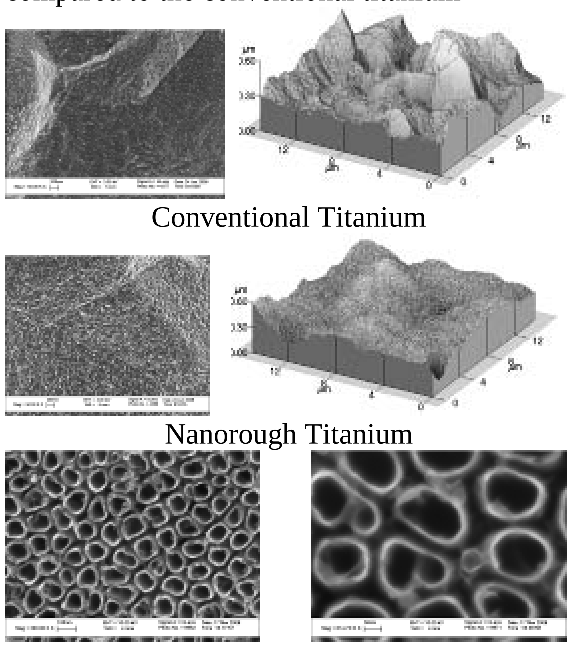

Figure 1. SEM and AFM images of conventional, nanotextured, and anodized nanotubular titanium surfaces.

CONCLUSIONS: Nanotextured titanium surface modifications created in this study by anodization and electron beam evaporation exhibited improved skin and bone growth. These observations suggest that nanotechnology techniques can create better medical devices thus deserving further investigation.

What can we learn about bone nano-structure from position-resolved small-angle x-ray scattering?

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INTRODUCTION: Small-angle x-ray scattering (SAXS) provides information on materials from the nanometer to the micrometer length scale. Since mature bone consists of an organic matrix with a high density of hydroxyapatite (HA) plate-shaped crystals with nanometer size, a lot of insight into the structure of bone can be achieved from SAXS.

About 15 years ago Peter Fratzl and coworkers[1] introduced a new dimension to SAXS investigations of bone structure by introducing position-resolved scanning SAXS (sSAXS). In this technique, a small beam with a diameter typically less than 100 microns in diameter is scanned across the sample. This gives additional information on the variation of the nano-structure of bone over macroscopic distances.

At laboratory based SAXS instrument one can obtain a lateral resolution of about 50 microns whereas intense synchrotron x-ray sources allow investigations with sub-micron resolution.

METHODS: We have used laboratory sSAXS for studying bone growth in trabecular bone in the vicinity of growth plates[1,2]. SAXS probes variation in electron density on the nanometer length scale independent of whether the material is amorphous or crystalline. Since the HA plate-shaped crystals have a high electron density, they contribute strongly to the SAXS signal. The sSAXS technique allows the crystal plate thickness and their orientation to be determined across the samples. In addition the degree of orientation within the probed volume can be determined.

RESULTS: In the mature trabecular bone, the HA crystallites are organized in a local lamellar structure with a characteristic texture around the holes in the trabecular structure. The traditional approach for analyzing the sSAXS, proposed by Peter Fratzl and co-workers, employs Porod's two-phase model. The approach does not exploit the full information content of the SAXS data and provides only the HA mineralite thickness under the assumption of 50% volume fraction of the platelets. We have used a new modeling approach with elements from scattering theory of colloidal systems as an alternative to the traditional approach. The model takes into account the local liquid-crystalline organization of the platelets and the full angle-dependence of the scattering intensity exploited by fitting the model to the SAXS data.

The analysis of the crystal orientation shows that a significant fraction of the platelets are oriented in the direction perpendicular to the growth plate in the calcified cartilage. The crystals in this region are thinner as compared to what is found in mature bone. Further away from the growth plate, the thickness increases with distance from the mineralization front and saturates at about 3 nm. The increase in HA plate thickness is accompanied by an increase in bone density, which is more pronounced in the calcified cartilage than further away from the growth plate.

DISCUSSION & CONCLUSIONS: The new approach for analysing the data from sSAXS measurements gives detailed and reliable information on the HA crystal plate thickness. The results indicate that new crystals are formed in the calcified cartilage whereas the increase in bone density further away from the growth zone is due to crystal growth.

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Designing Nanomedicines as Therapeutics and to Promote Tissue Repair

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INTRODUCTION: Convergence of interests in many different scientific disciplines relating to rationale design of nano-sized technologies for biomedical applications led to the birth of the field of 'Nanomedicine'. In turn this is giving rise to new materials and analytical tools for the improved diagnosis, prevention and treatment of diseases [1,2]. Everything we do in the field of nanomedicine is patient centric, and in this context, the 'nanomedicine**s'** include nanopharmaceuticals, nano-imaging agents and nanotheranostics (combination of imaging and therapeutic modalities). Over the last 2 decades there have already been >40 nanomedicine products approved for routine human use, and many more in on-going clinical development [1]. These pharmaceutical nano-technologies vary in size from 1-1000 nm and all have been rationally designed for a specific route of administration and to treat a particular disease.

TRANSLATION FROM LAB TO CLINIC: Our research has focused on the design of polymer therapeutics as nanomedicine**s** [3], and we designed the first synthetic polymer-anticancer drug conjugates to progress into clinical trials (1994) and also the first dendrimer-based anticancer conjugate (reviewed in [3-5]). Such conjugates were carefully (pharmacokinetically) designed to harness endocytic uptake for lysosomotropic delivery and to display either passive (EPR effect) or active (receptor-mediated) tumour targeting. The HPMA copolymer platform used initially had never before been tested in man. During this academic and industrial journey it became apparent that the key steps needed for successful clinical application of nano-technology in medicine include: i) a disease focus at the outset, ii) rationale design via interdisciplinary collaboration with leading edge technical expertise in **all** the core disciplines, iii) proactive assessment of nanomaterial safety in context of proposed use, and not least, an understanding from the outset of the needs for Industrial Development and Regulation Authority approval [6].

POLYMER THERAPEUTICS FOR TISSUE REPAIR: Having successfully transferred several families of HPMA copolymer anticancer conjugates into clinical trial (most recently HPMA copolymer platinates) [3,4], we began to explore a number of families of biodegradable polymer more suited to chronic administration and thus applications in tissue regeneration/repair. Clinical applications in age-related macular degeneration [7], arthritis and wound healing [8-10] were the goals of these initial studies. First it was necessary to define the endocytic properties of each target cell type in context of clinical use [11]. Using dextrin (degraded by α-amylase) and hyaluronic acid, (by hyaluronidase) as polymers to bind to proteins and peptides we also developed a new approach called 'PUMPT' [12]. Polymer conjugation is used to mask a protein's bioactivity and also protect it against degradation/inactivation in transit. Subsequent exposure to the activating enzyme at the target site leads to time-dependant reinstatement of protein bioactivity resulting in pharmacological response. Proof of concept studies and applications of PUMPT in wound repair are discussed here [8-10].

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Detection and characterization of nanoparticles in complex sample matrices

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INTRODUCTION: Nanotechnologies are forecasted to have a huge impact on all market segments, and new nanomaterials will be incorporated in a wide range of products and applications. But the same properties of the nanomaterials that makes them so interesting from application point-of-view can also give them toxic effects to humans or ecosystems. It has therefore been emphasized that one of the grand challenges for safe implementation of nanotechnology is the development of methods to monitor nanoparticles in workplaces, products and in the environment.

The main challenge for analysis and sizing of MNP in complex environmental samples is that there are extremely few MNPs but plenty of natural or unintentionally produced nanoparticles. It is difficult to detect particles present in extremely low concentrations among a large number of background particles with microscopic techniques such as TEM, and SEM.

METHODS: We have addressed this problem by further developing single particle inductively coupled plasma masspectrometry (spICP-MS) for detection and sizing of individual nanoparticles. spICP-MS is a real-time ultrafast scanning mode that allows capture of the ion burst events occurring when individual nanoparticles are vaporized, atomized and ionized in the plasma. The frequency of these ion bursts are directly proportional to the nanoparticle (of the specific element) number concentration in the sample. The spike height is proportional to the number of atoms (of the element) in each particle. Therefore in principle it is possible to obtain data on particle concentration and size (element mass) by the method.

Theory and fundamental aspects of the method will be discussed as well as opportunities and limitations that still need to be investigated or developed. Method developments using reference nanomaterials and standards will be shown.

RESULTS: When the dwell time are decreased (low ms) and approach the time of a particle ionization event (~0.1ms) then the signal to background increases proportionally since less acquisition of the background before and after the event contributes to the signal. The detection limit with respect to particle concentration is extremely low and depends on the analysis time (more data points yield better counting statistics for low conc). spICP-MS has previously been demonstrated for submicron particles and here we have estimated the detection limit in terms of smallest size that can be statistically distinguished from the background to be less than 10nm (based on gold nanoparticles with a sector field ICPMS). We have shown that there exists a linear relationship between the particle concentration and the number of particles counted. Our current research aims at developing the capability to size individual particles using the spike height. We are also developing methods for simultaneously measuring the particle bound, and ionic background concentrations of the elements.

In addition to use of the spICP-MS as a stand-alone method for screening nanoparticles in environmental samples (e.g. Ag and Ti in waste water), examples of using spICP-MS as an online detector following Field-Flow Fractionation will also be demonstrated for Pt and W in road runoff waters. The advantage of FFF is that the FFF provides the hydrodynamic diameter of the particles which in complex environmental samples often are different to the MNP size (the MNP may travel as part of a larger particle). Although a number of method aspects still need to be investigated, spICP-MS show great potential as a simple rapid selective method in nanoparticle research.

Induction of cellular responses by molecularly defined nanopattern

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INTRODUCTION: Integrin based adhesion has been shown to participate in numerous processes in living cells, which sense, via their adhesions, multiple environmental cues, integrate them, and develop a complex, multi-parametric response. However, due to their intrinsic molecular complexity the specific functional roles of different components of the adhesion site are still poorly understood *(1)*.

METHODS: The specific system investigated in this project is the extracellular matrix (**ECM**) adhesion system, whereby cells sense the chemical and physical properties of the surrounding environment, via specific receptors of the integrin family. The cellular responses to these interactions are complex and diverse, and include changes in the cells' shape and dynamics, reorganization of the cytoskeleton, and regulation of long-range and long-term signaling processes that affect cell viability, differentiation and proliferation (*1*). By working toward this aim, a specific molecular understanding of how living cells interpret complex chemical and physical cues presented to them by their immediate environment will be achieved. The stimulating environments used in these experiments will be either natural matrices, made of fibronectin- or vitronectin-, or synthetic matrices of varying degrees of stiffness or integrin ligand spacing, building on our past experience in nanoscopically defined model cell adhesion matrices (*2, 3)*.

RESULTS: Many cell functions are known to depend both on the elasticity of their environment and on the distribution of available cell adhesion ligands. So far an independent control of these two parameters in cell adhesion experiments *in vitro* has been impossible. Here we present a method which allows individual control of substrate stiffness, ligand density and spacing by fabrication of nanoscopically controlled biomolecule anchors using micelle nanolithography, followed by transfer onto elastic polyethleneglycole polymers. To evaluate cell adhesion on the substrates, live cell imaging and single cell force microscopy were used. We show that on surfaces with a Young's modulus larger than 8 kPa, cell adhesion can be manipulated by varying ligand density, whereas on softer surfaces cell adhesion is consistently deficient, irrespective of ligand presence (Fig. 1).

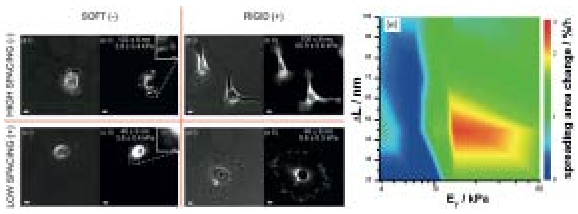

Fig. 1. Morphology of cells as affected by the physical state of the extracellular matrix *(molecular spacing and substrate stiffness as indicated by insets): Phase contrast (I) and fluorescence micrographs (II) of rat embryonic fibroblasts stably transfected with paxillin fused to yellow fluorescent protein (REF-YFP-paxillin) on elastic nanopatterned substrates under cell culture conditions 24 hours after seeding. Scale bars (a)-(d): 10 µm. Insets: Focal contact and focal adhesion formation on different substrates. Scale bars: 2 µm. (e) Signaling of cells as affected by variations of the physical state of the synthetic extracellular matrix (ΔL=molecular spacing of ligands, $E\gamma$=substrate stiffness): cellular spreading rate given in color code.*

DISCUSSION & CONCLUSIONS: Hence, our results demonstrate that substrate elasticity and ligand density is independent from each other with respect to cell adhesion response. It is also shown that the cellular spreading rate is maximized with physical substrate parameters that are between $\approx$ 12 and 30 kPa stiff, and 35-65 nm in ligand spacing presentation.

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Cell Road - Biomaterials for Neural Stem Cell Migration

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INTRODUCTION: The combination of nanotechnology and stem cell biology is one of the strongest hopes for regenerative medicine. This is particularly the case for neuro-degenerative diseases as well as stroke and brain trauma, since the brain has a limited capacity to replace dying neurons. However, a region of the adult brain contains a highly proliferative population of stem cells, which produces progenitor cells that migrate long-distance to the olfactory bulb[1,2].

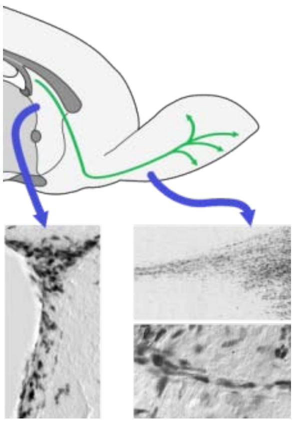


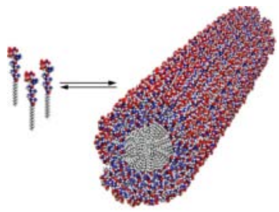
Fig. 1: Migration of neuronal progenitor cells from the subventricular zone to the olfactory bulb. Proliferating stem cells (left lower panel), migrating neuronal progenitor cells (right panels).

CONCEPT: The project explores the possibility of creating pathways for migration of neural progenitor cells to new target areas in the adult brain using biomaterials. The goal is to induce brain repair from endogenous stem cells by redirecting their migration through extracellular signals. Extracellular matrix proteins as well as cell adhesion molecules and cell membrane receptors can act as guidance and attractant molecules. The core of the current project is to find signal molecules and biomaterials that would allow redirection of the migratory pathway.

DESIGN: *In vitro model:* Using electrospun polyurethane nano- and microfibers we explore physical and chemical surface modifications to induce adherence and migration of neural stem cells.[3] The goal is to provide aligned matrixes that allow stem cell migration without significant changes in cell commitment, i.e. differentiation potential.

In vivo model: Peptide amphiphile (PA) molecules that self-assemble *in vivo* into supramolecular nanofibers will be used in an animal model to redirect progenitor cell migration. Because self-assembly of these molecules is triggered by the ionic strength of the *in vivo* environment, oriented nanoscale structures can be created within the extracellular spaces of the brain after liquid injection. The molecules are designed to form cylindrical nanofibers that present specific peptide epitopes to progenitor cells. Stereotaxic injection of the peptide amphiphiles will target the subventricular zone as the starting region and the substantia nigra, and neocortex as end zones.

Fig. 2: Peptide amphiphiles self-assemble to form nanofibers which are capable of providing a mechanical scaffold and biological signals to cells.



ANALYSIS TOOLS: Immunofluorescence, confocal microscopy, electron microscopy, quantitative PCR arrays, high content screening microscopy

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Peri-implant tissue colonization: "hide and seek" for biofilm bacteria

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Infection is one of the major causes of failure of inserted and implanted medical devices ("biomaterials"). The bacteria causing such infections are often commensals such as staphylococci.

The presence of a foreign body has essentially two different effects, (i) it is a surface to which bacteria can adhere, and (ii) it is a foreign body provoking a socalled foreign body response (FBR), The FBR is a well-regulated inflammatory response, initially having characteristics of an acute inflammation, but gradually changing into a more chronic inflammatory response, with novel tissue formation/encapsulation as the endpoint. Bacteria can profit from both these effects, resulting in formation of biofilms on the device surface, and in being less effectively cleared from tissue in the vicinity of the implant. The relative importance of biofilm formation and survival in peri-implant tissue likely depends on the type of device. In case the device is applied in flowing blood, biofilm formation will be very important. However, in case the device is implanted in non-flowing tissue, the peri-implant tissue will (also) be a potential reservoir for infecting bacteria.

Of these 2 elements of the pathogenesis of biomaterial-associated infection, biofilm formation has by far been the most extensively studied. Several approaches have been developed to provide antimicrobial capacity to biomaterial surfaces, either by inhibition of binding or by direct bactericidal activity.

Survival in host peri-implant tissue has been less well studied. It has been recognized that presence of a foreign body causes reduction of neutrophil phagocytic and microbicidal capacity (ref). It s also well known that staphylococcal infections of e.g. implants in bone will persist for long periods despite the removal of the implant and debridement, and that antibiotics are very inefficient in clearing these infections, despite the removal of the infected device.

We extensively studied biomaterial-associated infection with *Staphylococcus epidermidis* in a mouse model. Instead of only culturing the retrieved implant (as is often done) we also analysed the colonization of tissue, measured inflammatory mediators such as cytokines, and studied the histology. These studies showed that (i) bacteria were more often cultured from tissue than from the implants, and in higher numbers, (ii) some materials provoked a very strong inflammatory response in presence of bacteria, and others were anti-inflammatory, (iii) Bacteria were seen in large numbers in macrophages in the peri-implant tissue, at a distance of 10-20 cell layers away from the biomaterial-tissue interface, (iv) susceptibility to infection could be reduced by specific immunomodulation, (v) *S. epidermidis* in tissue resisted a regimen of rifampicin-vancomycin, and (vi) bacteria in tissue increased in numbers after several weeks, implying the tissue to be a reservoir for infection.

Subsequently, the presence of bacteria in human tissue was investigated, in deceased Intensive Care patients, who did not have a documented infection. From tisssue surrounding catheters staphylococci and enterococci were cultured, also from tissue not bordering the catheter. Bacteria were also detected within the tissue in immunohistology. So, also in humans tissue surrounding a foreign body is colonized by potentially infection-causing bacteria.

Biomaterial-associated tissue colonization and infection poses a number of novel research and clinical questions:

- How should biomaterials be developed with a low propensity to cause tissue infection?
- How should immunomodulatory strategies be developed to prevent/correct tissue infection?
- Are current antibiotic regimes sufficiently tailored to cover tissue, and to also kill intracellularly residing bacteria?

These questions will be discussed in view of better ways to prevent and treat biomaterial-associated infection.

Infection control on the biofilm level

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In any natural environment, macromolecules and micro-organisms have a strong tendency to associate with surfaces and form adherent microbial communities, so-called biofilms, which have been increasingly recognized as the cause of most infectious diseases. Biofilms develop on the surface of medical devices and cause implant-related infections but also on tissue surfaces where they give rise to caries, periodontitis, cystic fibrosis lung infections, otitis media, urinary tract infections and endocarditis. Much attention has been given to removal of detrimental biofilms, but commensal bacteria within biofilms of the skin, oral cavity and gut prevent the colonization of pathogens. A challenge is to enable treatment whilst maintaining the health-promoting functions of the commensal biofilm.

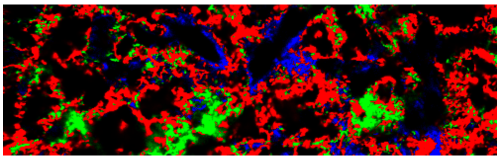

Figure 1: Multi-species biofilms colonising a rough titanium surface used for dental implants. 16S rRNA FISH was used to visualize streptococci (green), lactobacilli (red) and actinomycetes (blue).

The cardinal feature of biofilms of relevance clinically, is their distinct resistance to host defense mechanisms and conventional therapeutics, including many antibiotics and antimicrobial agents. Bacteria growing on urinary catheters can be up to 1000 times more tolerant to antibiotics than the same cells growing in planktonic culture.

Although the structural organisation and the colonizing micro-organisms at various locations are different, the establishment of a biofilm involves essentially the same series of well-regulated processes, including adherence to a proteinaceous film, co-adherence, cell division, metabolism and growth of micro-organisms. Detachment of adherent biofilm cells from the surface is generally regarded as a passive process in which bacteria are dislodged through physical forces but might also involve enzymatic activity of bacterial cells. The detachment of adherent cells serves as a means for the bacterium to spread to new sites.

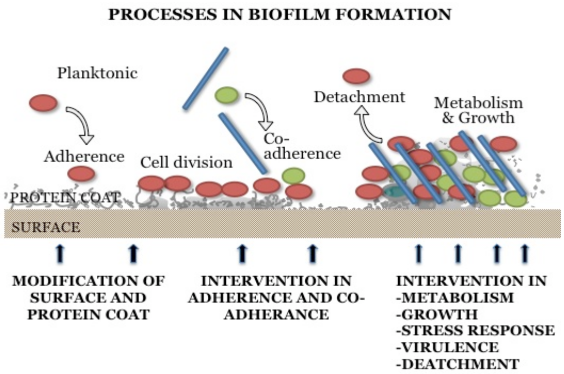

Figure 2: Possible strategies for biofilm control to prevent and treat infections.

A better understanding of processes taking place in biofilms is necessary for the development of novel strategies for infection control. Each of the processes that regulate biofilm formation presents potential targets. Since adhesion of microorganisms is strongly influenced by the nature of the substrate surface, one approach is to reduce the surface roughness or change the surface characteristics by manipulating the surface or the proteinaceous film. As the biofilm develops, antagonistic and synergistic interactions will dominate the microbial community. The biofilm matrix contains a multitude of micro-environments where the bacteria experience gradients *e.g.* in the concentration of nutrients, metabolic products, oxygen, growth factors and biocides. Such gradients are ¨sensed¨ by bacteria which respond and adapt possibly resulting in virulent and stress-tolerant phenotypes that would not exist in the planktonic stage. Consequently, in medical biofilms it is more appropriate to describe a biofilm by the microbial phenotypic and physiological properties rather than by the structure or composition.

Research questions that will discussed: How can bacteria change their phenotype during interaction with molecules of a protein coat? How do interactions between biofilm bacteria affect their virulence and resistance? How do bacterial cells “sense” and respond to surfaces and environmental change?

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Designing Electrospun Fibers for Tissue Engineering

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INTRODUCTION: Electrospinning is a straightforward method used to produce nanofibers from a wide variety of materials, including polymers, ceramics and metals [1]. It is a highly versatile method which can be used to carefully tailor many important properties of the spun fibers, from nano- to microscale [1, 2]. The work presented here shows some of the possibilities of designing electrospun materials to be suitable for tissue engineering applications, not least the possibility of creating highly porous scaffolds for cartilage tissue engineering [3, 4]. A new way of creating highly porous nanofibrous scaffolds investigated in this work was coating single microfibers with electrospun nanofibers [3, 4]. The nanofibers are then present to enhance cell adhesion and spreading, whereas the larger dimensions of the microfiber makes scaffolds of such fibers more porous with larger pores.

METHODS: Single PLA microfibers were coated with PCL or PLA nanofibers by use of electrospinning. Possibilities of creating hierarchical structures were investigated as fiber design was studied regarding nanofiber loading, diameters and alignment on the microfibers. Scaffolds with porosities of over 95% were made and seeded with chondrocytes to study cell infiltration.

RESULTS: The results showed that it was possible to collect nanofibers on single microfibers and use these in creation of scaffolds with porosities of over 95% (Fig 1) [3].

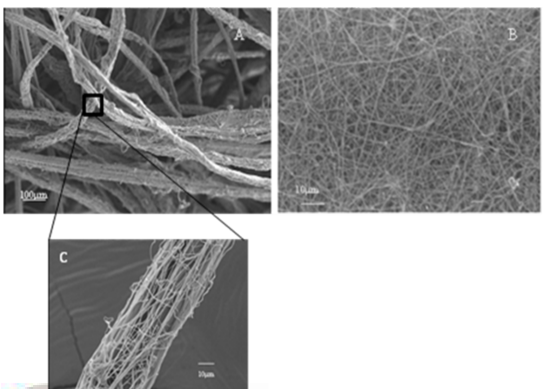

Fig. 1: Scaffolds of A) microfibers coated with nanofibers and B) only nanofibers. A magnification of a nanofibers-coated microfiber is seen in C.[3]

The nanofiber-coated microfibers could furthermore be designed in terms of nanofiber diameters, nanofiber alignment and nanofiber loading (Fig.2) [4]. This is of importance in tailoring of materials for different tissue engineering applications.

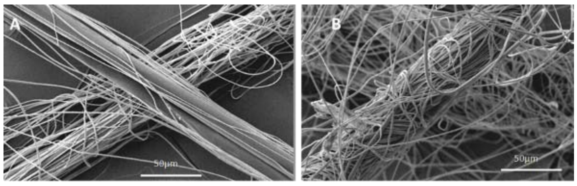

Fig. 2: Microfibers coated with A) aligned and B) random nanofibers.[4]

It was furthermore shown that chondrocyte infiltration into scaffolds of nanofiber-coated microfibers was greatly enhanced compared to the infiltration in pure nanofiber scaffolds [3].

DISCUSSION & CONCLUSIONS: This work shows that coating microfibers with nanofibers is a method with great potential in the design of functional scaffolds with very high control of architecture and topography, from nano- to macroscale, i.e. from nanofiber morphology to scaffold porosity.

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Why even difficult to avoid nanostructures in chemically bonded bioceramics?

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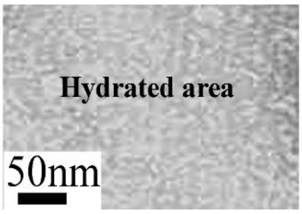
INTRODUCTION: The chemically bonded bioceramics (CBBC materials) comprise mainly phosphates, silicates and aluminates with calcium as the main cation. This presentation will give background aspects to the observed microstructures of the CBBC-materials, which all seem to exhibit nanostructures, both nanocrystals and nanoporosity. These bio-materials are now establishing their use as injectable biomaterials and as general bone void filler within dentistry and orthopedics, and as carriers for drug delivery. The presentation will focus on the chemistry behind. Most examples will be related to the potentially mechanically strongest and most acid resistant of the CBBC-systems, namely the Ca-aluminate (CA) based bioceramics.

METHODS: The surface and bulk com-position was analysed using thin-film XRD and SEM with EDAX. The microstructure was studied with HRTEM with samples prepared using focused ion beam microscopy (FIB) for high position site accuracy [1].

RESULTS: The CBBC-systems react in most cases in an acid-base reaction to form hydrates [2]. In water environment the CA material as an example, reacts at body temperature, as follows: 1) dissolution of CA into the liquid , 2) formation of ions, and 3) repeated precipitation of nanocrystals (hydrates) – Katoite, $CaOAl_2O_36H_2O$, and Gibbsite $Al(OH)_3$. The reaction involves precipitation of nano-crystals in the body on tissue walls and repeated precipitation until the CA is consumed resulting in complete cavity/ gap/void/ filling with low porosity. The nanocrystals (10-40 nm in size) bond to surrounding implant materials and to tissue (enamel, dentine and new bone). The materials property profile includes the following not so often simultaneous appearing and complementary properties related to nanotechnology; namely in situ, in vivo developed biomaterials with nanocrystals and nanoporosity with improved mechanical and integration properties.

DISCUSSION & CONCLUSIONS: The precipitation of all the nanophases is controlled by very low solubility products of the phases in the systems discussed [3]. The nanostructures of the CBBC systems based on Ca-silicates and Ca-aluminates include in addition to the principle phases of the main systems also a nanosize apatite phase when in contact with body liquid [4]. The alkaline systems transfer hydrogen phosphates in body liquid into pure phosphate and repeated pre-cipitation of apatite, $Ca_5(PO_4)_3(OH)$ occurs in the contact zone between the biomaterial and the tissue.



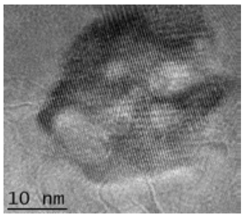


Fig. 1: Nanostructure of the hydrated CA-material (left), and precipitated apatite nanocrystal in the hydrated zone towards hard tissue (right).

REFERENCES: [1] Engqvist et al, Charac-terization of the tissue-bioceramic interface in-vivo using new preparation and analytical tools, *Adv. In Sci. and Techn. 2006; 49 p275-281* [2] Hermansson L, Kraft L, Engqvist H. Chemically bonded ceramics as biomaterials, *Key Eng. Mater.* 2003;247:437-442, [3] Axén et al, Zone formation at the interface between Ca-aluminate cement and bone tissue environment. *Ceramics, Cells and Tissues,* Proc. 9th Meeting, Faenza, Italy. 2004. [4] Hermansson L, Lööf J, Jarmar T. Integration mechanisms towards hard tissue of Ca-aluminate based biomaterials. *Key Eng. Mater.* 2009;396-398:183-186.

ACKNOWLEDGEMENTS: The author ex-presses his gratitude to all the Doxa personnel for valuable input under a ten year period.

Effects of nanosized hydroxyapatite coatings on osseointegration

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INTRODUCTION: For implants which are designed to integrate with bone tissue (osseointegration), the surface structure is very important to ensure rapid growth of bone cells and long term stability. Techniques to improve the osseointegration include roughening the surface with blasting or acid etching, or altering the surface chemistry with coatings such as titanium dioxide or hydroxyapatite (HA). We have developed a technique to coat the implant surface with an extremely thin (10-20 nm) layer of nanosized HA crystals. The coating can be applied on various implant materials, such as titanium, ZrO_2 and CoCr alloys, but we have also developed a technique to coat polymers such as PEEK.

METHODS: We use a self-assembling surfactant system to create a dispersion of nanosized HA crystals. For titanium implants, the dispersion is applied onto the implant by spin-coating, and a heat treatment for 5 minutes at 550 °C is done in order to remove the surfactants. This heat treatment also sinters the HA particles onto the surface. The size of the HA crystals, as measured with TEM and SEM, are approximately 20-40 nm long and 5-10 nm in diameter.

RESULTS:

An SEM image of a HA coated titanium implant surface is shown in Figure 1. As seen from this figure, the crystals are well-distributed and also follow the underlying substrate topography. XPS and EDS measurements show a Ca / P ratio close to the theoretical value of HA, 1.67.

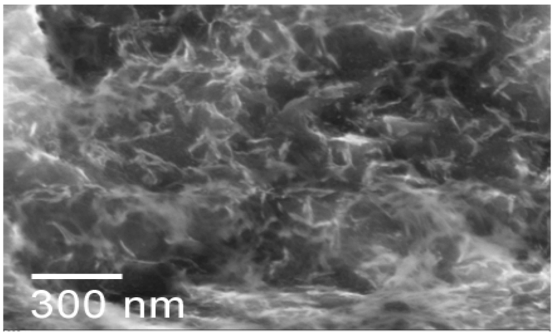

Fig. 1: Coating of nanosized HA on a titanium surface. Scale bar = 300 nm.

The effect of the coating has been verified in several in vivo studies. Shown in Table 1 are results from a study done on very smooth titanium implants, evaluated on rabbits after 4 weeks.

Table 1. Bone to implant contact values for coated vs. uncoated titanium implants.

Control (uncoated)	**Test (HA-coated)**
2.4 %	9.5 %

Another in vivo study evaluated the osseointegration of rough titanium implants with removal torque testing and with histomorphometry. The implants were removed after 2, 4 and 9 weeks. As seen in Table 2, the largest differences in removal torques are for 2 and 9 weeks.

Table 2. Removal torque values in Ncm for HA-coated vs. uncoated titanium implants.

	Control (uncoated)	**Test (HA-coated)**
2 Weeks	31	41
4 Weeks	35	37
9 Weeks	41	58

REFERENCES: L. Meirelles et al, Journal of Biomedical Materials Research A 2008, 87A, 2, 99-307
L. Melin-Svanborg et al, Int J Oral Maxillofac Surg. 2011 Mar;40(3):308-15.

Polymersomes for Drug Delivery

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Chemipilots A/S, Farum, DK

INTRODUCTION: Polymersomes are hollow capsules build by a block copolymer membrane [1]. Hydrophilic substances can be encapsulated into the aqueous core and hydrophobic substances into the lipophilic bilayer of the membrane of polymersomes (Fig 1, left). Polymersomes prepared from biocompatible polymers can be used as drug delivery vessels [2]. For most potential applications, small polymersomes (nm scale) are beneficial. A very important issue for potential drug carrier systems is the polymersome surface and its functional group.
In this work both small and large polymersomes (µm scale) were prepared from PLA-PEO block copolymers in aqueous media. Both PLA and PEO are known for good biocompatibility and are approved by the FDA for use in pharmaceuticals. Due to the properties of PLA such polymersomes will also be biodegradable.

METHODS: Amphiphilic block copolymers from PLA (polylactic acid) and PEO (polyethylene oxide) were synthesized by anionic polymerization. Small polymersomes prepared from these block copolymers were examined by cryo-transmission-electron-microscopy (cryo-TEM).
Fluorescent dye encapsulation into large polymersomes was observed by fluorescence microscopy and confocal laser scanning microscopy.

RESULTS: Small polymersomes (ca. 200 nm) and large polymersomes (ca. 5-10 µm) were prepared from PLA-PEO block copolymers. The polymersomes surface functionality was controlled by the choice of initiator for the block copolymer synthesis. The surface functionalization of polymersomes with amino-groups was shown by chemical attachment of a fluorescent reactive dye to the PEO-block terminal group. Large polymersomes prepared from a blend of functionalized and non-functionalized block copolymers showed a fluorescent ring in the microscope indicating polymersomes with dye attached to the membrane.

Encapsulation of both hydrophilic and hydrophobic substances into large polymersomes was shown with fluorescent dyes. 3D visualization of a large polymersome with a hydrophobic dye encapsulated into its membrane was realized with confocal laser scanning microscopy (Fig. 1, right).

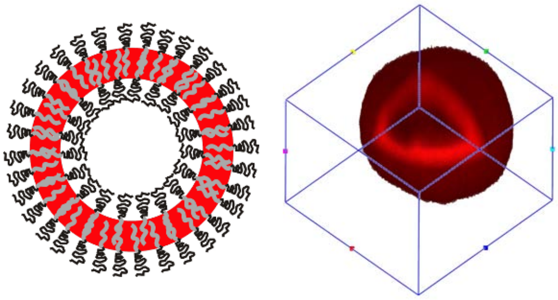

Fig. 1: Left: Schematic diagram of a polymersome with aqueous core (white) and lipophilic bilayer (red). Right: 3D visualization of a polymersome with encapsulated hydrophobic dye (Image from confocal laser scanning microscopy; scale:side line = 10 µm).

DISCUSSION & CONCLUSIONS:
If attaching a biological recognition group to the polymersomes surface instead of a dye specific targeting could be achieved. Encapsulation of drugs into such polymersomes would allow targeted drug delivery. Drug release from such polymersomes could i.e. be triggered by pH due to faster degradation of the PLA-block in low-pH media.

REFERENCES: [1] Antonietti and Förster, Adv Mater. 2003, 15: 1323-33. [2] Discher and Ahmed, Ann Rev Biomed Eng. 2006, 8: 323-41.

ACKNOWLEDGEMENTS: The described work was part of a PhD thesis carried out by the author at the University of Hamburg in the research group of Prof. S. Förster (2004-2008).

Nanobiomaterial – lipid membrane interactions

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INTRODUCTION: Novel nano-sized biomaterials are continuously being developed for a wide range of applications including drug delivery, *in vivo* imaging, *in vitro* diagnostics, and biomaterials for medical implants and tissue engineering scaffolds. In addition to common descriptors such as size, chemical properties and zeta potential, important characteristics of these materials can be understood by studying their surface interactions. Here, we present a methodology for *in vitro* characterization of nanobiomaterials based on their interaction with model lipid membranes, exemplified for cationic polyelectrolyte complexes[1, 2] (known to sometimes be cytotoxic) and graphene oxide[3] (non cytotoxic) (Fig. 1) based on complementary surface analytical techniques.

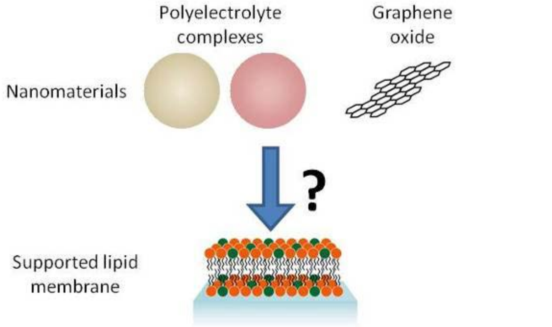

Fig. 1: Schematic illustration of the methodology presented (not to scale).

METHODS: Supported lipid membranes of two different lipid compositions (POPC:POPS (3:1) and POPC:POEPC (3:1)) were prepared by liposome rupture on SiO_2 surfaces.[4] Complementary information gained from atomic force microscopy (AFM) and quartz crystal microbalance with dissipation monitoring (QCM-D) is used. QCM-D real time interaction studies were performed in flow mode using an E4 instrument and AFM was performed in liquid using a PicoSPM microscope.[2]

RESULTS: Interaction studies between the cationic polyelectrolyte complexes and oppositely charged lipid membranes resulted in two separate outcomes. In experiments with oppositely charged membranes, the complexes generally collapsed onto the membrane forming a thin and rigid layer (Fig. 2A). Under certain conditions, the complexes adsorbed but were rinsed off extracting parts of the lipid membrane in the process (Fig 2 B). Graphene oxide adsorbed flat onto a positively charged lipid membrane, and was not removed by rinsing (Fig. 2C). Graphene oxide did not adsorb on negatively charged membranes.

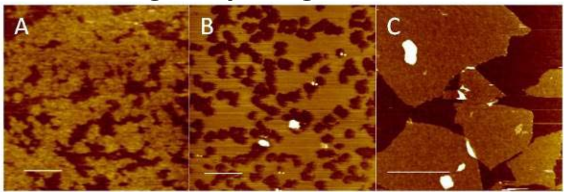

Fig. 2: AFM images of (A) cationic nanodrugs adsorbed to a lipid membrane[1], (B) holes in a lipid membrane observed after exposure to a cationic nanodrug and subsequent rinsing, and (C) graphene oxide adsorbed to a lipid membrane[3]. Scale bars equal 1 µm.

DISCUSSION & CONCLUSIONS: The studied nanobiomaterials are shown to adsorb onto or disrupt the lipid membrane. Although this data cannot be used to draw direct conclusions about the effect these materials have on cells, the results are in agreement with the general idea that cationic nanoparticles can be cytotoxic, whereas graphene oxide is not. In summary, the presented methodology provides a platform for further *in vitro* characterization of nanomaterials which can be used as a tool for screening and evaluation of nanomaterials. By using this methodology, the efficiency of further downstream testing, *i.e.* cell studies and *in vivo* trials, can potentially be improved.

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ACKNOWLEDGEMENTS: Financial support from NanoSphere (Formas) and the EU FP6 IP NanoBioPharmaceutics is gratefully acknowledged.

Nanoparticle encapsulation strategy improves biointerfacial properties of poly(ethylene glycol) hydrogels

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INTRODUCTION: Artificial tissues hold great promise for overcoming the setbacks of current tissue replacement therapies. To this end, we developed a nanoparticle encapsulation strategy that improves the permeability and patternability of poly(ethylene glycol) (PEG) hydrogel material. As a result, we demonstrate that this platform improves the viability and function of encapsulated human liver-derived cells, and also permits controlled, micron-scale spatial organization of encapsulated cells. Taken together, this design strategy offers an improved method for the development of more advanced artificial tissues that can promote high levels of cell metabolic activity and recapitulate key architectural features of human tissues.

METHODS: We synthesized hydrophobic PLGA nanoparticles with a mean diameter of 870 ± 34 nm. The mechanical properties of PEG hydrogel matrices were characterized with and without encapsulated nanoparticles. The viability and biochemical activity of human liver-derived cells and primary hepatocytes encapsulated into the hydrogel material were investigated. PDMS stamping was employed to pattern hydrogel structures.

RESULTS: Cell viability was increased for all measured thicknesses of nanoparticle-containing PEG hydrogel matries. We then tested the platform's potential for articial tissue engineering by encapsulating human adult primary hepatocytes that had lost a critical biochemical activity when they were cultured on collagen-coated 2D tissue culture plates. By contrast, we observed that this activity was restored in nanoparticle-containing samples. Taken together, these results show that incorporation of hydrophobic NPs is an effective way to improve phenotype stability of encapsulated cells.

Further, we tested whether this 3D environment was suitable for restoring advanced hepatic functions by adding fibroblast cells to the hydrogel material, thus providing both homotypic and heterotypic cellular interactions, as present in many tissues. The presence of fibroblasts on the matrix surface led to enhanced secretion of urea, as shown in comparison between samples. Morever, we established that cell patterning resulted in different levels of urea secretion (Fig. 1).

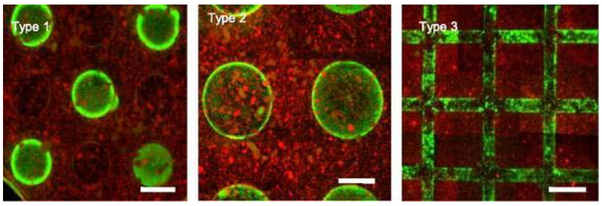

Fig. 1: Fluorescence images of encapsulated cells (red) on patterned PEG hydrogel matrices and patterned cells (green) on the surface. Hepatocytes and fibroblasts were encapsulated following 2-D coculturing. Scale bar: 1.0 μm

DISCUSSION & CONCLUSIONS: We incorporated hydrophobic nanoparticles into PEG hydrogel material in order to improve its biointerfacial properties, specifically permeability and patternability. The structural advantage achieved permitted us to construct micron-scale cell-trapping architectures for encapsulated cells to potentially restore heterotypic cellular interactions more akin to native tissues. We expect that this strategy to design network structures of cell-encapsulating hydrogels can be applied to restore more natural 3D environments of many tissues with complicated architectures.

REFERENCES: [1] Lee et al., *PNAS*. 2010 Nov; 107(48): 20709-14.

ACKNOWLEDGEMENTS: The authors wish to thank the Beckman Foundation, Burroughs Wellcome Fund, National Science Foundation, and the American Liver Foundation for supporting this work.

A simple methodological system for topographic and chemical nano-modifications of solid surfaces

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INTRODUCTION: A new and simple methodological system for the investigation of protein and cell interactions on nano-topographic and nanochemical modified surfaces will be presented

METHODS. *Adsorption of gold nanoparticles at gold surfaces* (1). Flat gold surfaces are modified with reagents so it become either positively charged or enriched with thiol groups (strong affinity to gold particles). Negatively charged 10–50 nm sized gold nano particles where adsorbed to the modified gold surfaces in buffers with various ionic strength.See Figure 1.

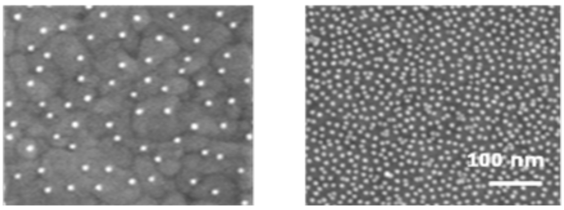

Fig 1. *SEM-photograph of 10 nm gold particles adsorbed on chemically modified flat gold surfaces. Left: adsorption was made in distilled water. Right: adsorption was made in high ionic strength. Eleectrostatic repulsion between the particles makes the difference in density.*

Sintered gold particle method. The gold nanoparticle decorated surface where washed

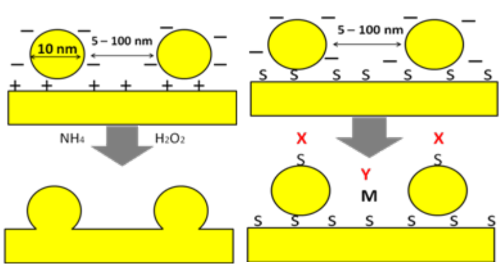

Figure 2. *Schematic illustration of the sintered gold particle method (left) and the binary chemistry modification method (right)*

a solution containing H2O2, and NH4. This treatment will sinter the gold particles to the gold surfaces and remove all reagents as judged from spectroscopic investigation with Tof-SOMS and XPS. The only difference between the flat and goldparticle surfaces will be the nanotopography. *The binary chemistry modification method (2)* The sulphur (thiol-containing) flat surface between the adsorbed nano-particles is modified with maleimide-tagged polyethylene glycol (PEG) making the surface between particles protein repellent, (M-Y) in Figure 2. The gold adsorbed gold particles is then modified with thiol containing reagents, e.g. thiols permitting cell attachements (S-Y) in figure 2. *The gradient method (3).* We have also developed a diffusion method for making a gradient of adsorbed particles with densly covered nanoparticles in one end and sparsely distributed particle at the opposite end. (Figure 3). The gradients are usually about 5 mm long. The gradient surface will greatly simplifye cell-adhesion research on the nano-modified surfaces.

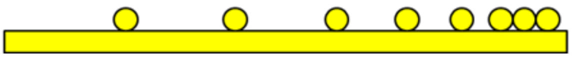

Figure 3. *The nano-particle gradient method*.

RESULTS. There are three papers at this meeting presenting results with the new methodological system:

***1.** Hulander M. et al* "Impact of nanostructure on bacterial adhesion and Extra cellular polysaccharide (EMP)"

***2.** Lundgren A. et al* "Nanoparticle Gradients for Investigation of Cell-Surface Interactions"

***3.** Berglin M. et al* "Hybrid nanoparticle arrays for measuring the interaction between cell adhesion macromolecules using SPR".

REFERENCES [1]Lundgren A. et al. *Nanoletters* 2008, 8, 3989-3992**:** [2]Lundgren A. et al. *Angewandte Chemie* int. ed. 2011 (in press) [3]Elwing et al *J Coll Inteface sci*. 119, 1 1987

Role of alumina nanoporosity in acute cell response

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INTRODUCTION: In the present work we studied the influence of biomaterial nano-topography on acute cellular response in an *in vivo* model. Biologically inspired materials are being developed with the aim of improving tissue integration and minimizing non-desirable host reactions. One promising strategy is to design topographically patterned surfaces that resemble those found in biological systems. Nanoporous alumina has been recognized as an important material and a template for the fabrication of nanostructures [1]. The effect of alumina nanopore-size on cellular responses has been studied in vitro, showing that pore size in the nanometer scale affects the extent of complement and platelet activation [2,3]. However, no study has been done so far to investigate the potential influence of nanoporous alumina surface topography on cell and tissue responses *in vivo*.

METHODS: Nanoporous alumina membranes with pore size diameters of 20 and 200 nm were fabricated by anodic oxidation of aluminium. To assess the acute inflammatory response to nanoporous alumina, 20 and 200 nm pore diameter membranes were implanted in the peritoneal cavity of mice. After 16 h implantation cell recruitment to the implant site was determined by fluorescence activated cell sorting analysis. Cell adhesion to the material surfaces was studied in terms of cell number, type and morphology by means of scanning electron microscopy (SEM) and immunocytochemical staining followed by fluorescence microscopy.

RESULTS: The *in vivo* study showed that 200 nm alumina membranes induced stronger inflammatory response than 20 nm membranes. SEM micrographs showed numerous adherent leukocytes on the 200 nm alumina membranes, many of which expressed signs of activation such as spread morphology. On the contrary, on the 20 nm alumina only traces of cell debris could be seen, i.e. no apparent attachment of cells on 20 nm alumina surfaces was observed.

Analysis of the immunocytochemical staining of the explanted membranes showed substantially more adherent cells on 200 nm alumina membranes than on 20 nm alumina implants, for both CD45+ and CD11b+ cells. The number of cells recruited to the implantation site also reflected the same tendency (Fig. 1).

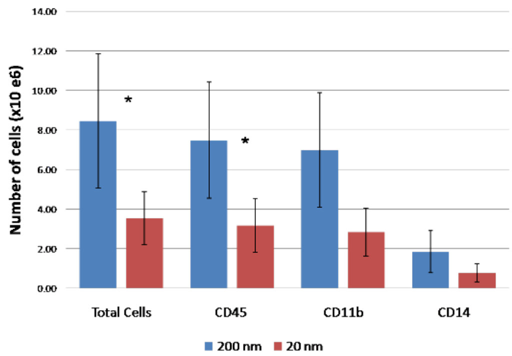

Fig1: Flow cytometry analyses of various cells in the peritoneal lavage solution recovered from mice after 16 hours of exposure to alumina membrane implants.

DISCUSSION & CONCLUSIONS: Since both pore-size membranes possess similar chemical composition, we believe that the observed difference in cell recruitment and adhesion is an effect of the material nanotopography. It is likely that alumina nanopore size exerts a direct effect on the acute inflammatory cells but also an indirect effect could take place where nanotopograhy affects protein adsorption in terms of amount, conformational changes, orientation, and exposure of cell binding sites. Our results suggest that nanotopography can be used to subtly control the recruitment and adherence of phagocytic cells during the acute inflammatory response to alumina membranes.

REFERENCES: [1] Yanagishita et al., Langmuir 2004; 20: 554-555, [2]Ferraz et al., J Biomed Mater Res 2008; 87A: 575-81. [3]Ferraz et al. J Mater Sci:Mater Med, 2008; 19:3115-3121.

Influence of Well-Defined Titanium-Coated Topographical Nanostructures on Bone Formation *in vivo*

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INTRODUCTION: Although the novel nanostructures at implant surface have been shown favorable bioactivities with titanium surfaces, there is still a lack of reliable data about the effect of nanotopography on bone response, because many other surface properties (chemistry, porosity, crystallinity) influence cellular interactions with these surfaces, and therefore it is difficult to draw conclusions and formulate general interaction principles between cells/tissues and nanostructured surfaces.

The objective of this study was to evaluate and compare the effects of different size but identical chemical composition nanoscale topographical features on osseointegration of titanium implants after 7 and 28 days of implantation in a rat model.

MATERIALS & METHODS: The test groups were machined cylindrical titanium implants modified with semispherical nanobumps of 60 nm, 120 nm and 220 nm diameter by colloidal lithography, and sputter-coated with 20 nm titanium layer to assure identical chemical composition of the surfaces. Sputter-coated machined titanium implants without nanobumps were used as a control. Surface properties of implants, such as chemical composition, topography and wettability were characterized with XPS, SEM, TEM, optical profilometry and contact angle geniometry.

80 implants were placed randomly into both tibias of 20 rats. Animals were euthanized 7 and 28 days postsurgery, and block biopsies were prepared for histologic, histometric and SEM analysis.

RESULTS: Differences between groups were found mainly in the new bone formation process, with larger reactions for the endosteal and marrow bone in the 60nm group after 28 days. Implant surface with 60 nm features demonstrated significantly higher bone–implant contact (BIC, 76 ± 14 %) compared with the 120 nm (42 ± 29 %) and control (47 ± 25 %) surfaces in endosteal and marrow bone compartments at 28 days. There were only non-significant differences in BIC between 220 nm (58 ± 6 %) and 60 nm surfaces.

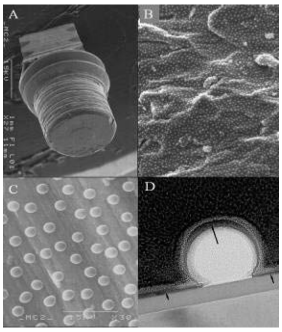

Fig. 1: SEM (A-C) and TEM (D) images of 220 nm semi-spheres patterned on cylindrical part of machined titanium implant.

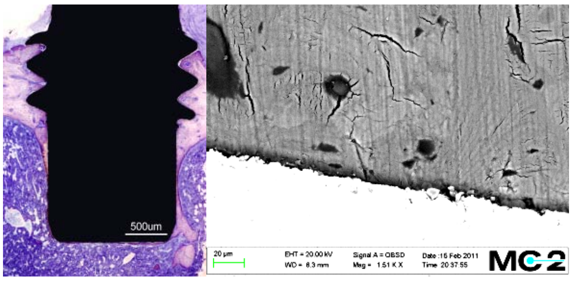

Fig. 2: Histological section and BSE micrographs of the 60 nm implant after 28 days healing

DISCUSSION & CONCLUSIONS: Our results confirm a phenomenon which is becoming increasingly apparent in the literature: nanoscale topographic features of biomaterials influence *in vitro* and *in vivo* bone formation.[1] Superposition of 60-nm semispherical nanostructures on the microscale topography of machined implant appeared to increase the extent of bone development after 28 days of healing in a rat model. We believe the effect is due to highest nanostructure density and surface curvature of 60-nm semi-spheres when compared to other nanostructures used in the study.

REFERENCES: [1]Christenson et al., J Orthop Res. 2007; 25: 11-22.

In vitro modeling of soft tissue infection; test model for new antimicrobials?

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Bacterial biofilms are known to play an important role in wound infection as well as biomaterial associated infections. *Pseudomonas aeruginosa* is a wound pathogen and well documented biofilm former that has been shown to delay the healing process in hard to heal wounds. *P aeruginosa* produces several virulence factors that kill host cells and destroy the tissues. It uses at least two different acyl homoserine lactone quorum sensing molecules (AHLs) to regulate their virulence. There is evidence in the literature that AHLs may inhibit the host response that may lead to prolonged infection without clinical signs of inflammation.
The objective here is to study interactions between clinical wound isolates of *P aeruginosa* and cultured human dermal fibroblasts and macrophages, the most important cells during wound healing.

Fibroblast cultured in 3D collagen matrices, and human macrophages adhered to a collagen surface, are treated with *P aeruginosa* biofilms, either as conditioned medium from established biofilm, or in co-culture with the biofilm itself. Cell viability and inflammatory responses is studied in correlation to the virulence pattern of the different clinical *P aeruginosa* strains.
Preliminary data show varying degrees of cell viability reduction after treatment with *P aeruginosa* biofilm/conditioned medium, however, not in clear correlation to the known virulence status of the different strains. The effect of AHLs and certain virulence factors on cell responses to bacterial stimuli is discussed.
The present wound modeling may be useful when testing new antimicrobial strategies aiming to disarm the virulent bacteria rather then killing the pathogens, a strategy with no risk for development of bacterial resistance.

A nano chemical debridement solution shows high efficiency on contaminated titanium surface

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INTRODUCTION: Peri-implantitis is an inflammatory process around an implant, characterized by soft tissue inflammation and loss of supporting marginal bone [2]. Regarding therapeutic measures several surgical methods are described in the literature, but so far there is no superior technique[1].The formation of a bacterial biofilm on the implants seems to be critical to the development of peri-implantitis. .[4]. Several reports demonstrate the healing potential of the peri-implant tissues after suppression of the peri-implant microbiota by mechanical and chemical means, however only limited scientific evidence is available to recommend any specific treatment modality. [3]. The aim of the study was to evaluate and compare different chemical agents for decontamination of titanium dental implants. The study was performed on polished titanium coins in order to standardize the testing surface.

METHODS:

S. epidermidis was allowed to form biofilm on the 53 polished and cleaned titanium coins (Ø=6.2 mm). First overnight cultures of 10 µl S. Epidermidis and 5 ml BHI were allowed to grow for 24 h at 37°C incubation in an aerobic atmosphere. Based on the result from a pilot study, these chemical decontamination agents was found suitable for further testing: sterile saline H_2O (VWR, Oslo, Norway), PrefGel™, (Straumann Institut, Basel, Switzerland).0.2 vol% Chlorhexedine, 3 vol % H_2O_2 (VWR, Oslo, Norway) and a mixture of 3 vol% H_2O_2 and 1.6 g/L nano TiO_2 powder (P25 Aeroxide, Degussa Evonik, Evonik Industries AG, Essen, Germany. Evalutation of the chemical removal of the biofilm was assessed in SEM and with UV-Vis spectroscopy.

RESULTS:

SEM image of decontamination with sterile saline water show no effect on the surface (Fig. 1), where there is no change in the biofilm. Similar finding was found for 0.2 vol % chlorhexidine (Fig 1, B). SEM image of decontamination with PrefGel™ showed noticeable breakage of the biofilm layer (Fig 1, B), but still large amount of remaining bacteria. Images of decontamination with 3 vol% H_2O_2 and the mixture of 3 vol% H_2O_2 and 1.6g/L nano TiO_2 powder showed a marked visible difference compared to the other solutions (Fig 1, E). UV-Vis analyze showed significant ($p>0.05$) lower amount of bacteria left after decontamination with 3 vol% H_2O_2 and 1.6g/L nano TiO_2 compared to all other solutions (data not shown).

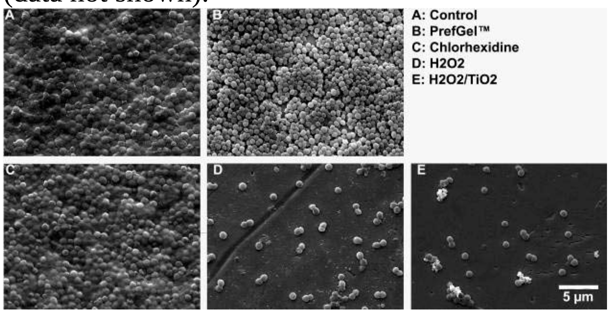

Fig. 1: SEM images of S. Epidermidis biofilm on titanium surfaces after de-contamination with various chemical solutions

DISCUSSION & CONCLUSIONS:

According to this study the solution of 3 vol% H_2O_2 and 1.6g/L nano-TiO_2 significantly debridded the titanium surface better than the all other tested solutions. Chlorhexidine, PrefGel ™ and saline water had no/minor effect on the biofilm on the titanium surfaces and thus showed very little decontamination effect. **REFERENCES:**

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*These authors have contributed equal amount into the work and should therefore all be regarded as first author

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Photocatalytic Activity of Low Temperature Oxidized Ti-6Al-4V

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INTRODUCTION: Photocatalysis on TiO_2 surfaces by UV illumination has been studied and applied in a range of fields, including biomaterials for its known bactericidal effects [1]. TiO_2 irradiated with UV light generates an electron-hole pair, which reduces O_2 and oxidizes H_2O to superoxide ions (O_2^-) and hydroxyl radicals (•OH), respectively [2]. These can then decompose nearby organic material, such as adherent bacteria. The current study is a preliminary investigation on photocatalytic activity of TiO_2 surfaces obtained by a quick, low temperature surface modification technique, using H_2O_2 and H_2O as oxidizing agents. The aim is to optimize the oxidation process and apply the active surface on dental Ti-6Al-4V crowns and bridges produced via electron beam melting (EBM). This would add an on demand, *in situ* antibacterial feature to the device, reducing bacterial pressure and avoiding the onset of peri-implantitis.

MATERIALS AND METHODS: Discs of Ti-6Al-4V were manufactured from EBM rods (Arcam AB, Mölndal, Sweden). H_2O_2 treatment at 80°C for 30, 75 or 120 minutes was followed by hot water (80°C) aging for 90, 225 or 360 minutes. The effect of long-term oxidation (18h in H_2O_2, 54h in H_2O) was also investigated. Photocatalytic activity after oxidation was determined using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). Discs were placed in a flat-bottomed cuvette filled with 5µM of the colored, organic solution rhodamine B (rB). A small well drilled in the bottom of the cuvette allowed magnetic stirring of the dye during tests. Pulsing (100 Hz) UV light at 365 ± 10 nm was irradiated from 2 cm above sample surfaces. Degradation of rB due to photocatalysis on TiO_2 was monitored by stepwise absorbance measurements at 5-minute intervals for up to 8 hours. Oxidized surfaces were imaged using a LEO 1550 SEM (Zeiss, Oberkochen, Germany).

RESULTS: A clear trend between oxidation time and photocatalytic activity can be seen in *Figure 1*, where absorbance (related to rB concentration) is plotted as a function of time under UV irradiation. Approximating the degradation rate of rB as a first order reaction, a rate constant can be derived from each curve. Preliminary results show a near linear behaviour between oxidation time and photocatalytic activity when comparing rate constants from short- and long-term oxidations.

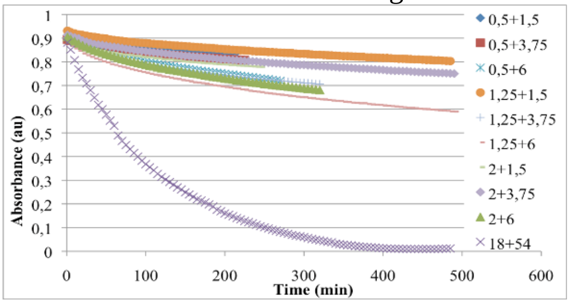

Fig. 1: Degradation of rhodamine B over time. Legend indicates sample oxidation time in H_2O_2 and H_2O, respectively.

Figure 2 shows a clearly distinguishable TiO_2 surface layer only after long-term oxidation, which further explains its higher photocatalytic activity.

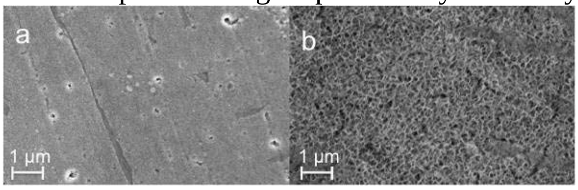

Fig. 2: SEM images of samples oxidized for a) 2+6h and b) 18+54h in H_2O_2 and H_2O, respectively.

DISCUSSION & CONCLUSIONS: The presented surface modification technique of Ti-6Al-4V is a cheap and straightforward way of obtaining photocatalytically active surfaces with on demand antibacterial properties. TiO_2 growth and photocatalytic activity shows strong dependency on oxidation time, and results suggest a linear relationship. Long-term oxidation is necessary to achieve desired antibacterial effect, and further studies will reveal optimal oxidation parameters.

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SOLID-STATE NMR, A SOLUTION FOR STRUCTURE ELUCIDATION

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Even though its solution-state counterpart is more widely used in elucidation of chemical structures, solid-state NMR spectroscopy has become increasingly important for analytical chemists. With hardware development, solid-state NMR resolution is now comparable to that of solution-state NMR [1]. In addition, phenomena which are not detectable by solution-state NMR, e.g. anisotropic nuclear spin interactions, can be studied or effectively utilized with solid-state NMR, giving rise to new possibilities when designing NMR-experiments [1].

A wide range of information can be acquired using solid-state NMR. In studies of cellulose; degree of crystallization, fibril and aggregate size, and relative amounts of cellulose allomorphs (Iα, Iβ, II etc) can be investigated [2, 3], as seen in Fig. 1 [4].

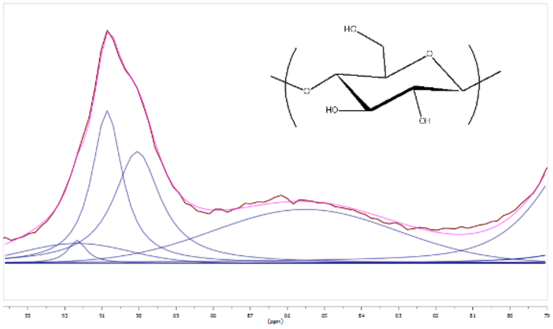

Fig. 1: Spectrum (red) showing the C-4 area from a sample of nanocrystalline cellulose. Line-fitting (blue, pink) gives information about degree of crystallization, fibril and aggregate size, and relative amounts of cellulose allomorphs.

As a tool for elucidating crystal structures the technique has become invaluable and when studying conformation, bond angles and bond lengths, solid-state NMR shows benefits concerning the ability to analyze inhomogeneous and amorphous samples, compared to traditional methods such as XRD [1].

When studying metal-ion coordination, solid-state NMR gives information about coordination location [5] as seen in Fig. 2 [6]. Furthermore, T_1-relaxation experiments can provide additional information about coordination location as well as information about rigidity and surroundings.

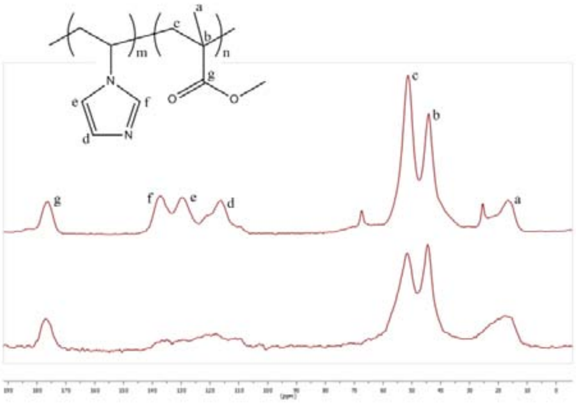

Fig. 2: Solid-state NMR spectra of a copolymer before and after addition of Cu^{2+}. The addition affects the peaks corresponding to the imidazole group more than the ester functionality, implying that the metal coordinates to the former part of the copolymer.

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Nanoparticle Gradients for Investigation of Cell-Surface Interactions

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INTRODUCTION: Surfaces with nanoscale features have successfully been used to improve the understanding of adhesion-mediated environmental sensing of cells. We recently described how small nanoparticles can self-arrange with tuneable separation on gold substrates [1] and how this can be used to form large chemical nano-patterns for functional cell studies [2]. We have also found a method for making the nanoparticles self-assemble into gradients with high concentration of particles at one end and low at the other end of a surface. We expect this to greatly simplify experiments on the mechanisms of cell-surface interactions.

METHODS: The surface preparation method is outlined in figure 1 below. Nanoparticle gradients of different steepness were made on gold substrates using a new generic approach that will be presented. Some gradients were made on surfaces suitable for SPR (Surface Plasmon Resonance) analysis. The gradients were modified with PEG between the particles and polymers that promote endothelial cell growth on top of the particles as shown in [2]. The gradients were characterized with SEM, Dynamic Contact Angle and imaging SPR [3]. Human Endothelial cells were seeded on the gradients and cell number/morphology was investigated after 24h by microscopy.

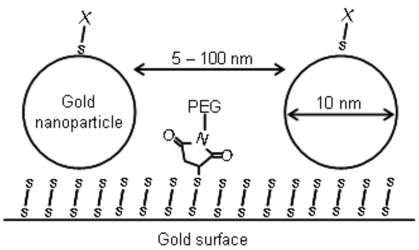

Fig. 1: A gold surface is functionalized with octanedithiol and 10 nm gold particles are then adsorbed to the surface. The degree of separation is tuned by electrostatic repulsion (Debye-screening) between the negatively charged particles. Maleimide–PEG is added and will react with the free thiols forming a protein repellent surface between the particles. A functionalized thiol or macromolecule is then adsorbed to the gold particles.

RESULTS: An iSPR image of a 4-mm gradient is shown in figure 2, revealing that the gradient is smooth and uniform. Also protein adsorption on top of the gradient was measured by iSPR (not shown). A micrograph of the gradient with adsorbed cells is shown in figure 3. A transition in the amount of cells can be seen at a surface coverage of 10% corresponding to a particle separation of 30 nm (red line). The spreading (size) of the cells however changes in proportion to the particle density.

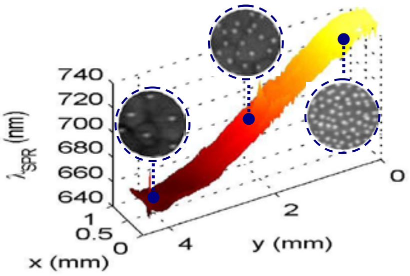

Fig. 2: Nanoparticle gradient visualized with imaging SPR. The SPR wavelength (z-axis) is proportional to the particle surface density. The insets show particle density determined with SEM.

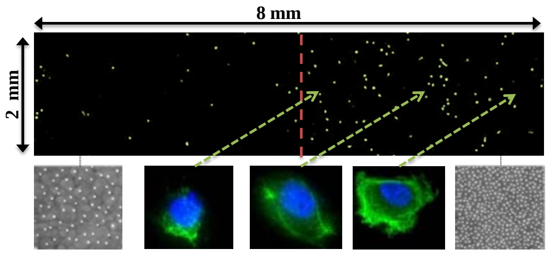

Fig. 3: Endothelial cells (small dots) on a gradient of polymer modified particles on a PEG-background. Insets show cells in higher magnification at different positions.

DISCUSSION & CONCLUSIONS: The transition in cell number indicates a threshold for cell adhesion at this surface composition. The cell spreading is however not correlated to the attachment, but to the number of particles. This may not be easily detected without the use of a gradient. The gradient may thus help us to gain new knowledge in different, but related, areas of cell-surface interaction such as cell adhesion, role of ligand-receptor interactions, cell proliferation and cell migration.

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Effects of a Natural Oil on the Mechanical Properties and Cytotoxicity of PMMA Bone Cement

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INTRODUCTION: Vertebroplasty, using poly (methyl methacrylate) (PMMA) –based bone cements, is an effective treatment to stabilize spinal fractures and provide pain relief. However, this method has been associated with additional fractures next to augmented vertebrae, possibly due to stress shielding effects [1]. Hence, injectable, low-modulus bone cements are preferred and have been investigated in different studies [2]. The introduction of pores via an aqueous phase is one way to reduce the modulus of the cements. However, this resulted in an excessive particle release *in vitro*, probably due to incomplete polymerization of powder entrapped in the aqueous phase [3]. To avoid this effect, the addition of a natural oil component to the PMMA was evaluated in this study. Castor oil was chosen, as it is a polar oil, soluble in the MMA monomer and is generally considered non-toxic [4]. The resulting cements were investigated in terms of the mechanical and the cytotoxic effects of the addition of oil.

METHODS: In this study a commercially available bone cement, Osteopal® V (Heraeus Medical GmbH, Germany), was used. Different concentrations of castor oil (Sigma-Aldrich, USA) were incorporated into the cements during the mixing process. The curing and mechanical properties were assessed according to ASTM F451. The cytotoxicity of extracts was investigated according to ISO 10993-5. Extraction was carried out in culture medium for 24 h at 37°C. To mimic the conditions during application of the cements, the medium was added 2.5 minutes after the beginning of mixing. MG-63 cells were cultured in 24 well plates at a concentration of 30000 cells/cm². One day after plating, the culture medium was replaced by the conditioned media obtained as described above. Cell viability was assessed after 1 and 3 days using the alamarBlue® viability assay (Invitrogen).

RESULTS: The maximum polymerization temperature decreased significantly with any addition of oil. Furthermore, both the compression stiffness and the maximum compression stress were found to decrease with an increase in the amount of oil added. A statistically significant difference (ANOVA, $p<0.05$) was found between all groups for the mechanical properties. The viability of cells cultured in extraction media was lower compared to cells maintained in normal culture medium. Moreover the viability tended to be lower when higher amounts of oil were added to the formulation. This was also accompanied by a change in cell morphology (Figure 1).

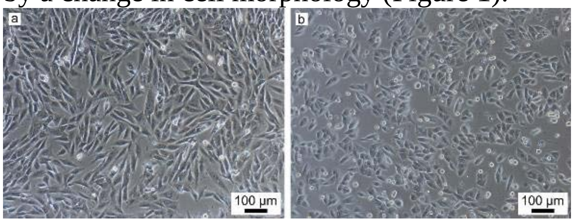

Fig. 1: Cell Morphology of MG63 cells after 1 day of cultivation in normal culture medium (a) and extraction medium from PMMA + 12 wt% castor oil (b)

DISCUSSION & CONCLUSIONS: The addition of castor oil to PMMA bone cement results in many apparent advantages such as reduced polymerization temperature and lower stiffness. The oil is likely to act as a plasticizer within the cement. However, the oil leads to an increased cell toxicity of extracts, maybe due to enhanced release of monomer residues. Thus, further additives have to be tested which prevent cytotoxic effects of extracts while lowering the modulus of the resulting material.

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Porous biodegradable coatings for drug delivery

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INTRODUCTION: Water droplets were used as templates for pores in biodegradable poly(hydroxybutyrate), PHB, coatings made from water-in-oil emulsions. The amount of water and size of the water droplets in the emulsion template determines the degree of porosity and the creation of pore networks of the resulting film coating. A pore network in a coating will enable the diffusion of hydrophilic drugs through the coating layer. Coating materials were designed with multiple layers and different degrees of pore network formation.

METHODS: Films and coatings were prepared by solution casting by solvent evaporation of single and multiple layers in Petri dishes. The porous PHB coating material was prepared using a water-in-oil emulsion template technique. A typical preparation is as follows: 560 mg PHB was dissolved in 8 ml of chloroform at 58°C during agitation for 30 min. 100 µl of 1.5 % (w/v) Span80 in chloroform was added to the PHB solution. A solution of 2.86 % (w/v) Li_2SO_4 in MilliQ water was prepared. 800 µl of Li_2SO_4 solution was injected to the PHB solution with a Hamilton syringe during homogenization. Pure PHB films and coatings were prepared by dissolving PHB in chloroform without homogenization. The water permeability was studied using a diffusion chamber and tritiated water according to a method described previously [1].

RESULTS: Fig 1 shows the cross sections of a selection of porous PHB prepared by emulsion templates. Films made from an emulsion with only 5% water content have pores of about 0.5-1 µm (see Fig 1b). An increase in water content to 10% (see Fig 1a) results in pore sizes of about 0.5-2 µm and increased interconnectivity.

The water permeability was determined for coatings made from pure PHB films and films made from water-in-oil emulsion templates containing 5-10 % water. Films made from pure PHB and emulsions of 5-7 % water were practically impermeable whereas a film made from 8% proved to be slightly permeable with a water diffusion of about $3.3 \cdot 10^{-12}$ m^2s^{-1}. The permeability increased to $2.5 \cdot 10^{-11}$ and $8.7 \cdot 10^{-11}$ m^2s^{-1} for 9 and 10% water, respectively. The permeability of the coatings consisting of multiple layers was controlled by the layer having the least porosity.

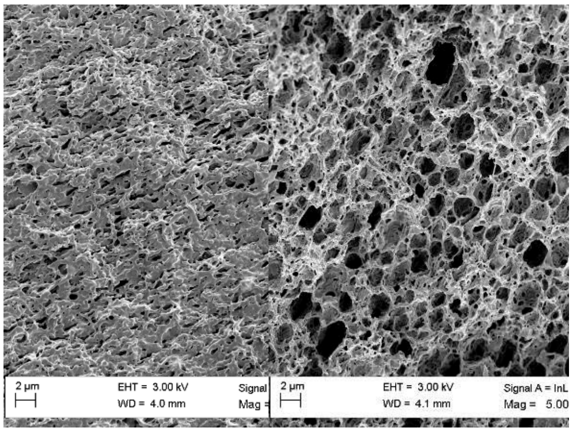

Fig. 1: Freeze fractured cross sections of PHB coating material consisting of: a) 10 % water-in-oil emulsion (to the left); b) 5 % water-in-oil emulsion (to the right).

DISCUSSION & CONCLUSIONS: PHB coatings with varying degree of porous interconnectivity have been produced by a water-in-oil template method. The amount of water in the water phase of the emulsion template determined the droplet distribution and droplet size in the resulting film. The coatings made from emulsions of 5-7% water content were almost impermeable which suggests that the pores are not interconnected. The water permeability and pore interconnectivity of the coatings increased with the water content of the template emulsion. The diffusion through the multiple layer coatings was controlled by the porosity and layer design.

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ACKNOWLEDGEMENTS: Financial support was kindly provided by the Vinn Excellence Centre SuMo Biomaterials (Supermolecular Biomaterials – structure dynamics and properties) and Chalmers Area of Advance – Materials Science.

Premixed calcium silicate cements for endodontic applications

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INTRODUCTION: Ceramic cements based on calcium silicate are commonly used in endodontics due to their good sealing ability, biocompatibility and bioactivity. During injection of the cement, filter pressing may lead to a reduction in calcium silicate content and strength of the final product. This could be avoided by using a premixed paste, where the aqueous phase has been exchanged for glycerol[1]. In addition, a premixed version of the cement has superior clinical handling characteristics. However, to the authors' knowledge, there are no reports on the effect of common variables on the properties of these types of cements, based on calcium silicate. The present study consisted of three parts. Firstly, the effects of liquid to powder ratio (L/P), amount of radio-opacifier and accelerator were screened using a statistical model *(I)*. Secondly, L/P was optimized for cements containing three different amounts of radio-opacifier *(II)*. Finally, the effect of using glycerol rather than water was evaluated in terms of radio-opacity *(III)*.

METHODS: Portland cement (Ca_3SiO_5) was manually mixed with different amounts of radio-opacifier (ZrO_2) and accelerator ($CaSO_4{\cdot}1/2H_2O$). The resulting powder was then mixed with glycerol. The injectability was assessed using a Compules® Tips Gun with 20G needle tubes. The paste was considered fully injectable (FI) if the gun and needle tubes could be used and injectable (I) if injectable through a 1ml syringe. The specimens were stored in Dulbecco's Phosphate Buffered Saline solution at 37°C and the setting time (ST) was assessed using the Gillmore needle method. The radio-opacity of the cements was assessed using 1mm high specimens under X-rays at 50kV and 3mA.

RESULTS: The amount of accelerator did not have a significant effect on the results. An increase in amount of $ZrO_2$was found to give an increase in ST. The L/P ratio and the amount of ZrO_2 were both found to significantly affect the radio-opacity (Figure 1). The results of study part II are shown in Table 1. For part III, it was found that specimens mixed with water had a 13% higher radiopacity than those mixed with glycerol.

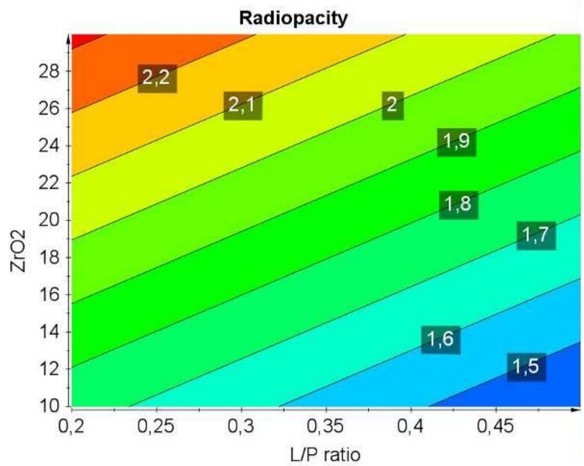

Fig. 1:Effects of the amount of radiopacifier and L/P ratio on the radiopacity of the cements.

Table 1.Results of Part II.

ZrO_2 [wt%]	***L/P [ml/g]***	***Initial ST [h]***	***Final ST [h]***	***Radiopacity [mmAl]***
20	0.28	7.2±0.9	8.4±0.7	1.8(0.1)
25	0.26	6.8±1.0	8.1±0.2	2.1(0.2)
30	0.24	6.8±0.9	8.6±0.2	2.3(0.2)

DISCUSSION & CONCLUSIONS: The use of glycerol permitted low L/P ratios. The increase in STs with the amount of radio-opacifier was likely due to the higher density of ZrO_2 in comparison to Ca_3SiO_5, which gave a higher L/P in terms of volume. In fact, after optimising the L/P for different amounts of ZrO_2, similar setting times were observed (Table 1). The decrease in radio-opacity with an increase in L/P was probably due to the higher amount of porosity obtained with a higher L/P. The long setting times found with the glycerol are acceptable for use in applications such as root fillers, where the cements are injected into a confined space with a low risk for washout.

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Growth and viability of *Staphylococcus epidermidis* on UV irradiated and fatty acid-coated smooth titanium surfaces

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INTRODUCTION: Infection is a problem in implant surgery, leading to complications after implant placement. Bacteria in biofilms on implant surfaces are more resistant against antibiotic treatment than are non-attached bacteria, a problem that is further increasing with resistance against antibiotics. It is therefore desirable to produce surfaces that reduce or even prevent bacterial attachment or that reduce their proliferation. Smooth titanium (Ti) is a very common material for use as temporary implants like bone fracture plates.[1] UV irradiation is used frequently for sterilization and has been claimed to be the reason for enhanced biocompatibility [2] and increased in antibacterial activity.[3] Also the n-3 fatty acid eicosapentaenoic acid (EPA) has shown antibacterial activity.[4] In this study, we compared smooth Ti surfaces with UV treated surfaces and surfaces coated with EPA [5] for early attachment of *S. epidermidis*.

METHODS: Ti disks (cp.grade 4) were mirror polished and washed.[5] The samples were sterilized in 70% ethanol before UV irradiation for 48h. Some of the samples were subsequently coated with a thin layer of chemically adsorbed EPA. Non-modified smooth Ti sterilized in 70% ethanol served as a control.

S. epidermidis culture was done on freshly prepared Ti surfaces. The disks were placed in 48-well plates (Nunc) on ice and 500 µl of bacteria culture in BHI medium with an OD_{560} of 0.03 was added per well. The samples were incubated at 35°C in ambient air for 16h.

For quantification of the biofilm coverage, the bacteria were stained with 0.1% safranin solution after fixation in Sørensen's phosphate buffer containing 2.5 % glutaraldehyde. The OD_{560} of acetic acid with the detached biofilm was measured.

To quantify the amount of dead bacteria, the biofilms were stained with Hoechst 33342 (HOE; all bacteria) and propidium iodide (PI; dead bacteria) and fixed as above before examination with fluorescence microscope and quantification with ImageJ (A_{PI}/A_{HOE} x 100%).

RESULTS: The total area covered with bacteria was slightly influenced by the different surface modifications (Table 1); the amount of bacteria on EPA-coated surfaces was significantly reduced compared to surfaces that had been UV irradiated for 48h. On Ti, the percentage of dead bacteria was lowest, while higher ratios of dead bacteria were measured for all the other surface treatments tested.

Table 2: Total biofilm area and percentage of dead bacteria.

	Ti	UV	UV+Eth	EPA+UV
Biofilm area [%Ti]	100.0 ± 23.8	115.8 ± 35.6	95.4 ± 29.2	83.6[a] ± 20.0
% dead b. [% Ti]	100.0 ± 81.6	251.77[b] ± 83.1	428.4[b] ± 157.2	374.3[b] ± 247.2

(a – $p<0.05$ *vs.* UV, b – $p<0.05$ *vs.* Ti)

DISCUSSION & CONCLUSIONS: Bacterial films were found on all surfaces tested, however, the percentage of dead bacteria was significantly increased for the various surface treatments. Treatment of Ti surfaces with UV light and EPA coating resulted in higher percentage of dead bacteria which might indicate an environment less favourable for bacterial growth. The results of the present study show that there might be a significant influence of surface chemistry and wettability properties after this surface treatment on the biofilm viability of *S.epidermidis*.

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ACKNOWLEDGMENTS: The help of Sébastien Taxt-Lamolle and Jessica Lönn-Stensrud with the bacteria culture is greatly acknowledged.

Composite silica gel as test bed for flow in nano porous materials

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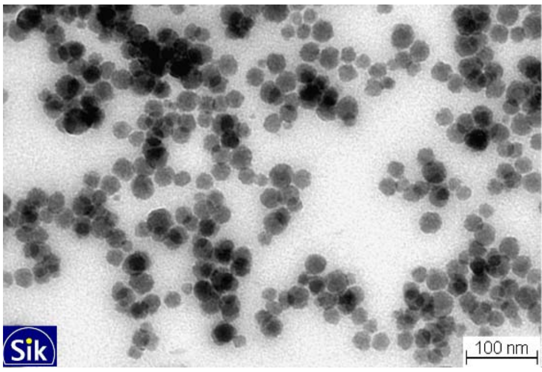
INTRODUCTION: The scope of the study is to design and construct soft model materials to investigate how material nano and micro structure control diffusion and flow of solutes and gases within biomaterials. By precise design of porosity and surface characteristics general lessons will be learned for better design of materials used in drug release, implants, packaging and paper industry. Internal structure of biomaterials can be varied in an infinite number of ways; however, some materials are more suitable when requiring variable and highly defined structures and geometries that are straightforward to image and model. Colloid silica is a material that fulfils many desirable characteristics of a model material. The silica forms gel networks that can be tailored and by adding nano cellulose the permeability can be further refined.

The project is part of SuMo Biomaterials that is a VINN EXCELLENCE centre financed by VINNOVA. The center is an academic and industrial joint effort with a clear focus on understanding and developing properties of soft biomaterials.

METHODS: The gels was prepared by the sol gel method with the silica sol Bindzil 40/130 consisting of 20 nm SiO_2 spheres. Nano crystalline cellulose was synthesised by sulphuric acid hydrolysis[1] and characterized by AFM, SEM, XRD, and XPS. Gelling was done by varying silica concentration (0-10 wt%), pH (4-8), salt concentration(0-1M) and in some cases adding nano cellulose ($1*10^{-4}$ - $1*10^{-3}$ wt%). Diffusion is to be measured in the gels by 1H NMR and structure of gels was mapped by TEM tomography. Gels were embedded in polymer and microtomed prior to TEM imaging.

RESULTS: Phase diagrams showed that as low silica concentration as 3 wt% gave mechanically stable gels and that the gelling can be tuned to minutes or weeks. Gels was possible to image in TEM, *Fig. 1*.The addition of small amounts of nano cellulose (*Fig. 2*) did affect the sol stability and gel formation.



FiFig. 1: TEM image of cross section of colloid silica gel

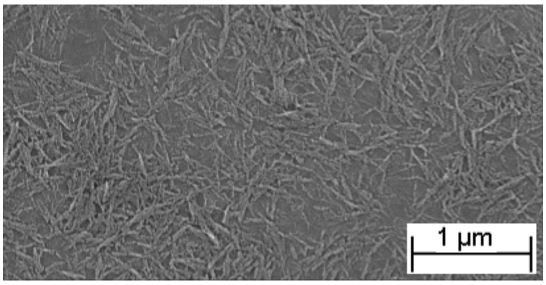


Fig. 2: SEM image nano cellulose spin coated on a silica surface and sputtered with gold.

DISCUSSION & CONCLUSIONS: Phase diagrams have mapped interesting regions for further investigation with NMR and TEM. Initial TEM images shows promising results and 1H NMR will soon be used to measure diffusion. The gels provide a promising model material and test bed for new theories about mass transport in hierarchical structures.

REFERENCES: [1]CD. Edgar and DG. Gray , Cellulose, 2003 10(4): 299-306, DOI: 10.1023/ A:1027333928715

ACKNOWLEDGEMENTS: The authors gratefully acknowledge VINNOVA, AstraZeneca, Bohus Biotech AB, Eka Chemicals, Lantmännen, Mölnlycke Health Care, SCA Hygiene Products, SIK AB, Södra Cell AB, Tetrapak for the providing of research funding within the SuMo Excellence centre.

Enzymatic mineralization of gellan gum hydrogel for bone tissue engineering and regeneration

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INTRODUCTION: Gellan gum (GG) is an anionic polysaccharide widely used in the food and pharmaceutical industries. Recently it has been used as a hydrogel biomaterial for cartilage regeneration [1]. However, for application in bone tissue engineering, it shows shortcomings typical for hydrogels, namely low stiffness and insufficient mineralizability. Hydrogel mineralization can be achieved by addition of enzymes that catalyse deposition of bone mineral, such as alkaline phosphatase (ALP) [2]. In this study GG hydrogels were functionalized with ALP to determine the amount and composition of the calcium phosphate mineral phase formed within the hydrogel matrix.

METHODS: GG hydrogel cylinders were prepared as described previously [1]. ALP was incorporated at 50°C. Four sample groups with ALP concentrations ranging from 0 to 2.5 mg/mL gel were produced. Samples were incubated in 0.1M calcium glycerophosphate (CaGP) at 22°C or 37°C with daily medium change. After 2 or 6 days the samples were washed with double-distilled water (Milli-Q), weighed, dried and reweighed. Solid weight percentage, i.e. the gel weight percentage consisting of polymer and mineral and not water, was calculated. Mineralized samples were analyzed by SEM, EDX, FTIR and XRD.

RESULTS: The solid weight percentages of GG cylinders containing different concentrations of ALP incubated for 2 and 6 days in CaGP at 22°C are shown in Fig. 1. The solid weight percentage increased with incubation time and with ALP concentration. Higher incubation temperature (37°C) did not increase solid weight percentage of the samples (data not shown). SEM images of the samples with ALP show mineral-like deposits, while EDX analysis revealed the presence of calcium and phosphorus (Fig. 2), which were absent in controls. In FTIR spectra bands characteristic for phosphate groups were detected. XRD showed that mineral deposits were fine-crystalline hydroxyapatite with crystallite sizes of approximately 14 nm.

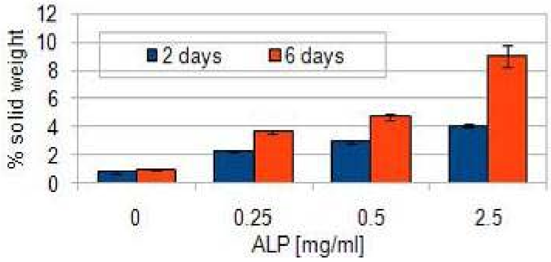

Fig. 1: Solid weight percentage of GG with different ALP concentrations incubated for 2 and 6 d in CaGP.

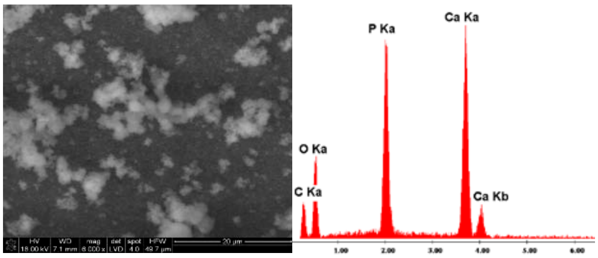

Fig. 2: SEM (left) and EDX (right) of GG containing 0.25 mg/mL ALP after 6 d in CaGP.

DISCUSSION & CONCLUSIONS: The results indicate that ALP incorporated in GG retained its activity and, when incubated in CaGP, induced formation of fine-crystalline hydroxyapatite within hydrogel matrix. The materials produced according to this approach seem to be promising as composites for bone tissue engineering.

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ACKNOWLEDGEMENTS: The authors thank AgentschapNL, the Netherlands, (IOP Self Healing Materials, Project no. SHM08717) and Polish Ministry of Science and Higher Education (Project no. NN507280736) for financial support.

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Analysis of *Staphylococcus epidermidis* biofilm formation on nanostructured hydroxyapatite surfaces

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INTRODUCTION: Biofilm formation on medical devices is a common cause of implant failure, especially regarding implants that breach the epithelial tissue, so called transcutaneous implants. The key issue to prevent such infections is to create a direct bonding between the implant and the soft tissue and to eliminate the bacterial adhesion and biofilm formation on the material used. Nanotechnology and the development of new nanomaterials have given the opportunity to design nanotextured implant surfaces. Such nanosurfaces have been shown to benefit bone cell growth[1] and in a recent study, nanotextured titanium surfaces were shown to enhance keratinocyte proliferation and spreading[2]. However, little is known about how bacteria interact with nanostructured surfaces. The aim of the present study was to investigate how nanostructured hydroxyapatite (HA) coated surfaces effect *Staphylococcus epidermidis* proliferation and biofilm formation in vitro.

METHODS: Three differently shaped nano-HA particles: coral, rice and rod (Promimic AB) were spin coated onto titanium discs and characterized with x-ray photoelectron spectroscopy (XPS), and scanning electron microscopy (SEM). The three different nano-HA coated surfaces, TiO_2 and polytetrafluoroethylene (PTFE) discs were tested in a biofilm assay using safranin staining. Simultaneously, the antimicrobial effects of the different coatings were evaluated by measuring the optical density of the planktonic cells. Different microscopy techniques were also employed to visualize the biofilm (results not shown here).

RESULTS: Scanning electron microscopy and XPS confirmed the presence of nano-HA particles on the titanium discs (fig. 1). The preliminary results of the biofilm assay from two independent experiments performed in quadruplet indicated no difference between TiO_2 and the different nano-HA coated surfaces in biofilm biomass formation. However, all surfaces experienced 10 times larger biofilm biomass formation than the negative control surface, PTFE. The OD measurements of planktonic cells did not indicate any antimicrobial properties of the surfaces.

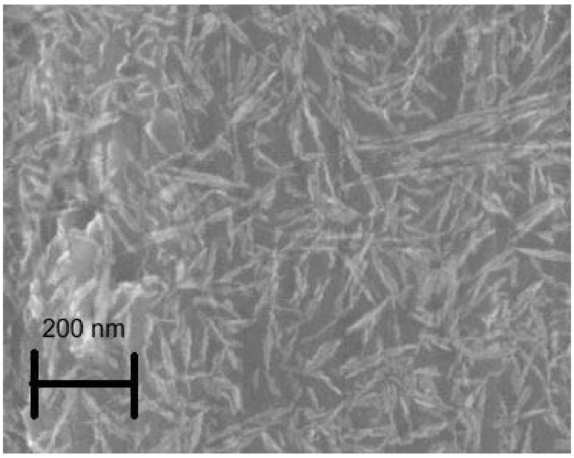

Fig. 1: Scanning electron micrograph of rod shaped nano-HA particles spin coated onto TiO_2 disc.

DISCUSSION & CONCLUSIONS: The results of the biofilm assay with safranin staining indicated no significant difference in biofilm formation between the nanostructured surfaces and the conventional TiO_2 disc. In conclusion, it seems as if nano-HA particles coated onto TiO_2 does not exhibit increased biofilm formation compared to plain TiO_2 even though the specific surface area is much larger for the nano-HA coated surfaces than the conventional TiO_2.

REFERENCES: [1]Colon et al., Jour. Biomed. Mat. Res. A. (DOI: 10.1002/jbm.a. [2]Puckett et al., Acta Biomaterialia. (DOI: 10.1016/j.actbio.2009 .12.016).

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Real-time characterization of biomaterial compatibility using Quartz Crystal Microbalance with Dissipation monitoring (QCM-D)

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Q-Sense

INTRODUCTION: When designing artificial biomaterials to be used in the field of orthopedic implantology or stents it is of crucial interest to ensure a low level of immune response and avoiding infections caused by these materials.

The Quartz Crystal Microbalance with Dissipation monitoring technique (QCM-D) enables monitoring of the adsorption at a solid-liquid interface in real-time. QCM-D can successfully be used to provide information on molecular adsorption processes with various surfaces *via* the quantification of parameters such as mass, thickness, viscosity and shear modulus of the sensor surface adhering film. Here, we review a study where the technique has been used to obtain information on biocompatibility of artificial biomaterials.

RESULTS: [1] The study reported here is addressing the problem with blood clot formation in stent implantation (antithrombogenicity) and endothelialization (adsorption of endothelial cells). The authors deposited an anticoagulant heparin and cell compatible collagen in a layer-by-layer (LbL) fashion. The properties of this multilayer were examined in respect to its antithrombogenicity and endothelialization. QCM-D and other optical techniques such as electron microscopy were used to determine that the formed multilayer was smooth and stable. In summary, the study showed this surface coating to have great potential in facilitating *in situ* endothelialization of blood contacting materials.

DISCUSSION & CONCLUSIONS: This poster highlights a recent advance within the field of biomaterials and how QCM-D can be used as relevant real-time tool for such investigations.

REFERENCES:[2] Lin Q., et al., *J Biomed Mater Res A*, **2010**, (epub ahead of print)

ACKNOWLEDGEMENTS: This poster was modified with kind permission from the authors.

A Novel Wear Model Simulating Abrasion on Composite Dental Filling Materials

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INTRODUCTION: The use of composite/glass ionomer fillings has had a world-wide increase due to restrictions and prohibitions on the use of amalgam, as well as patients' growing demand for aesthetics even in posterior regions [1] Wear is a multi-factorial phenomenon that results in loss of material. In the case of composites, wear is defined as loss of anatomic contour [2]. There is at present no standard method for testing wear of dental composites [3]. The aim of this study was to establish a wear model for testing filling composite materials with abrasion properties closer to a clinical situation. In addition, the model was used to evaulate the effect of filling volume and particle size on surface roughness and wear resistance.

METHODS: The incisors were prepared with 9 wells, each with a round diamond bur (d = 0.5 mm) (Figure 1, 1). Each molar (n=4) was acid-etched and bonded to each other with Adper™ Single Bond Plus Adhesive (3M Espe, Oslo, Norway).

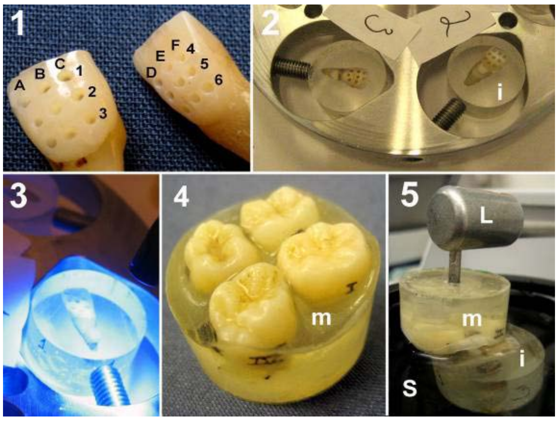

Fig. 1: (1) Test incisors with wells, (2) Placement of incisors imbedded in epoxy block (i) (3) acid etching and curing with LED of the composite prior to grinding and polishing, (4) The human molar cusps served as antagonist to simulate wear and were fixated in epoxy (m) (5) The resin block (i) with the test composites were placed in human saliva (s) and fixated. The lever (L) conducted circular randomized movement of block (m) onto block (i).

Generic composite of 3 different filler volumes and 3 different particle sizes hold together with the same resin were randomly filled in respective cavities. Evaluation was performed in microCT, SEM and profilometer

RESULTS:
The surface roughness, fractal dimension and core fluid retention index were found lowest in the group of medium filling content and smallest particle size ($p<0.05$). A trend in volume loss and optical evaluation in SEM also support these findings.

Fig. 2: SEM images of selected wells after the simulated wear procedure. The average particle size is smaller than for LM, MM and HM.

DISCUSSION & CONCLUSIONS:
The model is believed by the authors to be closer to an *in vivo* wear situation than the other reported wear simulations models. The antagonist peaks of the cusps had detectable wear to the enamel, but did not reach the dentin, although worn for 120 000 wear cycles. The present study suggest, contradictory to previous studies, that the most wear resistant filling materials should consist of medium filling content, and that particles size is not as critical as earlier reported.

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[1]Burke FJ, J Dent 2004 Jul;32(5):343-350. [2]DeLong R, Dent Mater 2006 Aug;22(8):702-711.[3]Heintze SD, Dent Mater 2006 Aug;22(8):712-734.

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An *in vitro* evaluation of mesoporous titania implants

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Introduction: The aim of this study was to evaluate mesoporous cubic and hexagonal titanium dioxide implants. The surfaces ability to initiate hydroxyapatite formation was assessed using an *in vitro* method, which is based upon simulated body fluids (SBF). The mesoporous implants were compared to non-porous.

Method: Mesoporous and non-porous titania thin films were prepared via an evaporation induced self-assembly process.[1] The implants were evaluated using three types of simulated body fluids (SBF); one containing dissolved salts that simulated the composition of human plasma, one with salts and albumin and one with salts and alendronate (a well-known osteoporosis drug).[2] The adsorption of ions, albumin and alendronate were monitored using quartz crystal microbalance with dissipation monitoring (QCM-D) and the hydroxyapatite formation was studied as a function of time using SEM, TEM, FTIR and XRD.

Results: In Figure 1 SEM micrographs of the formed implant surfaces are presented, a) hexagonal, b) cubic and c) non-porous titania. For the cubic mesoporous titania, 6 nm pores can be seen.

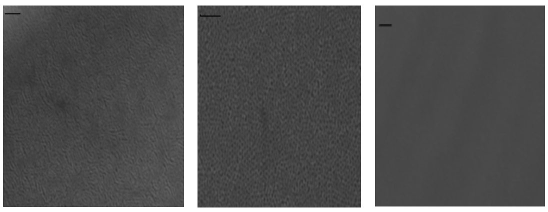

Figure 1: SEM micrograph of a) hexagonal, b) cubic and c) non-porous titania surfaces (scale bar equals 100 nm)

Results obtained from the QCM-D measurements are presented as the change in frequency plotted as a function of time (Figure 2). As can be seen, there was a higher change in frequence, corresponding to a higher ion adsorption, on the hexagonal and cubic mesoporous surfaces compared to the non-porous surfaces. In addition, the presence of protein and alendronate decreased the ion adsorption, particularly on the non-porous surface.

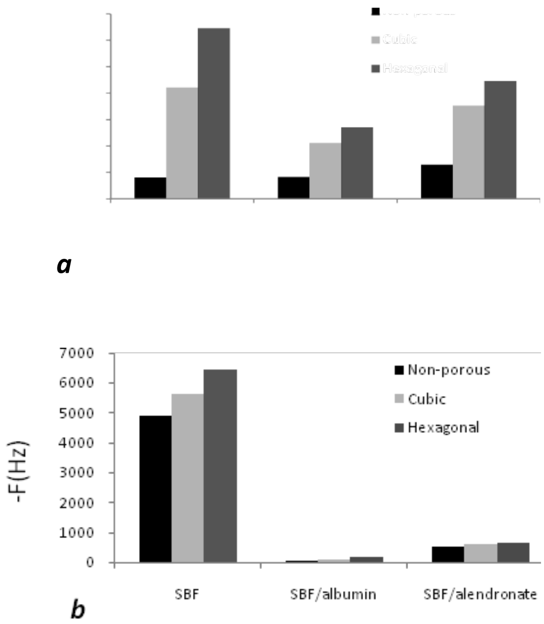

Figure 2: QCM- results after a) five hours b) 40 hours on hexagonal, cubic and non-porous surfaces in SBF, SBF/albumin and SBF/alendronate.

Discussion and conclusion: The QCM-D results correlated well with the rate of hydroxyapatite formation, which was monitored using SEM, TEM, XRD and FTIR. The combined results clearly show that the presence of pores increases the formation of apatite.

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2. Oyane, A., et al. Journal of Biomedical Materials Research Part A, 2003. **65A**(2): p. 188-19

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MG63 Osteoblast-Like Cell Responds to Nano Pits and Nano Bumps

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INTRODUCTION: Nano-fabrication technology combined with cell studies has opened new ways to investigate the mechanisms of cell attachment, growth and differentiation on the surfaces in the last few decades[1]. Model surfaces with controlled chemical and topographical features at nanoscale can be produced to simplify the experimental system, which enables to isolate and study their interactions with specific components in the biological system. In this study we are aiming to compare biological reaction of MG63 cells cultured on a flat control surface and topographically nano-structured surfaces containing bumps or pits.

METHODS: Colloidal lithography was used as nanofabrication technique[2]. Positively pre-charged glass substrates were exposed to the colloidal solution of negatively charged polystyrene nanoparticles (150nm diameter). The adsorbed and dried polymeric nano spheres were heat treated in an oven to form semi-spherical shapes (nano-bumps). In order to make nano pits, a 65nm thick layer of Ti was evaporated on the glass surfaces containing adsorbed polymeric nano spheres. By tape-stripping off the nano spheres, 65nm deep nano pits were formed into the Ti layer. Finally, a 10-nm thick Ti layer was sputtered on the nanopatterned surfaces to make the surfaces chemically homogenous. The same Ti layer was sputtered on flat glass surfaces to serve as reference surface.

MG63 osteoblast–like cells obtained from human osteo-sarcoma were cultured on the fabricated and flat surfaces in presence of vitamin D_3 for up to 7 days. Cell attachment, morphology and viability were analyzed after 2 days of culture. Cell proliferation and alkaline phosphate activity were characterized after 7 days of culturing. Additionally, surfaces and cells were imaged by SEM both after 2 and 7 days of culturing.

RESULTS: Cell number determination on different substrates didn't show significant differences in 2-day cultures, however cell proliferation after 7 days was significantly less on nano bump patterned surface in comparison with the other tested surfaces. The cells on nano bumps showed significantly higher ALP production per cell compared to other surfaces although total ALP activity levels of the cells on the different surfaces were similar.

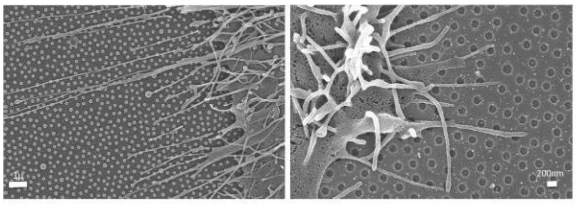

Fig. 1: Filopodia of MG63 cells in interaction with nanobumps (left) and nanopits(right).

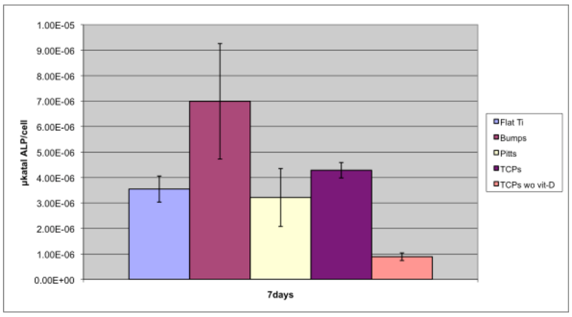

Fig. 2: ALP activity of the cells in response to different surfaces in 7 days culture.

DISCUSSION & CONCLUSIONS: Proliferation of the cells on the nano bumps was less intensive, while ALP activity per cell was significantly higher than on the other surfaces, suggesting that 150nm bumps have clear synergistic effect with vitamin D_3 stimulation on the cell differentiation. The finding may contribute to the development of novel bone implant surface modifications.

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Osseointegration of mesoporous implants

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INTRODUCTION: Development of new implants with improved technologies to stimulate bone formation is of great interest in the biomedical field. Addition of a mesoporous film on the implant could bring improved osseointegration. One reason could be the change of surface topography for the implant, and the other reason could be its ability to serve as a drug-delivery system, using active substances stimulating bone growth.[1] The aim of this project was to coat implants with mesoporous TiO_2 thin films, and investigate if the surface topography of the mesoporous coating can have a positive affect for osteogenesis at the bone tissue/implant interface. To evaluate this, an *in vivo* study was performed using implants that were inserted in rabbit tibia.

METHODS: Mesoporous TiO_2 thin films were synthesized using the evaporation induced self assembly (EISA) method.[2] Implants were coated with mesoporous and non-porous (used as reference) TiO_2 thin films, using spin coating. To characterize the samples TEM, SEM, XRD, XPS, nitrogen adsorption and contact angle was used. The implants were inserted in rabbits and the study proceeded for three and twelve weeks. At each time point, nine rabbits were used for removal torque measurements and one rabbit for histology evaluation.

RESULTS: Characterization using TEM showed that the mesoporous TiO_2 films were ordered in a cubic arrangement, and that the pore size was 6 nm.

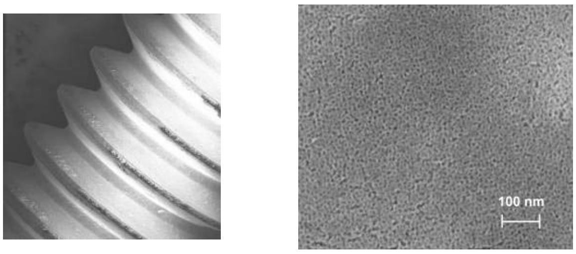

Fig. 1: SEM micrographs, of an implant coated with a mesoporous TiO_2 thin film.

In SEM, it was seen that homogenous thin films were obtained on the implants, Fig. 1 (left). At high magnification in SEM, the pores were visualized, Fig. 1 (right). For the *in vivo* study, removal torque results for three weeks have been evaluated, Fig. 2.

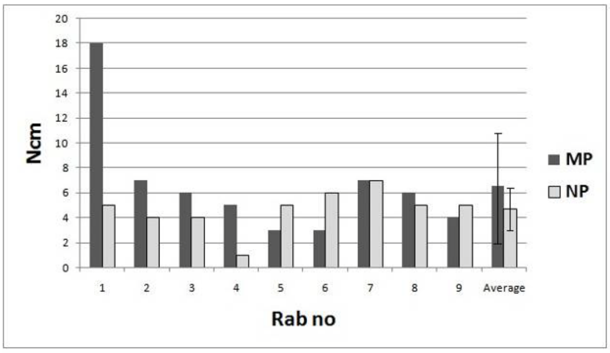

Fig. 2: The removal torque after three weeks for mesoporous and non-porous (reference) samples.

Measurement for the twelve weeks removal torque and histology for both three and twelve weeks are still under investigation.

DISCUSSION & CONCLUSIONS: Mesoporous TiO_2 thin films were successfully prepared having a cubical arrangement and a pore size of 6 nm. After three weeks there was no significant difference in osseointegration between the mesoporous and the non-porous samples. Since the mesoporous TiO_2 thin films did not bring a negative affect considering the osseointegration, it would be of interest using them for drug-delivery purposes.

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ACKNOWLEDGEMENTS: The authors would like to thank the Materials Area of Advance at Chalmers University of Technology for providing financial support.

A dental cement capable of calcium phosphate formation on its surface during water storage

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INTRODUCTION: In the field of biomaterials research, bioactivity is a well known concept and a thoroughly investigated feature. A bone bioactive material has the ability to form a bond with living tissue. However, in dentistry (with the exception of dental implants, for which several explants can be offered) it has received little attention, plausible due to the general properties of the current dental materials. A dental cement with a combined setting reaction has been proven to show *in vitro* bioactive properties [1], i.e. formation of hydroxyapatite on the surface when stored in PBS. This is speculated to give an increased durability in the bond between the prosthetic device and tooth substance. In this work, the potential ability of the cement to form apatite on the surface during storage in water was investigated. Such feature would be important since there would then be no need for saliva for apatite formation to occur *in vivo*.

METHODS: The material investigated in this study was Ceramir® Crown & Bridge (CB) (Doxa AB, Sweden), a dental luting cement that mainly consists of calcium aluminate mixed with a glass ionomer. The CB powder was mixed with a liquid phase in a powder to liquid ratio of 3.2 and allowed to harden for 10 minutes at 37°C and >90 % humidity in rubber moulds (8 mm in diameter and 3 mm in height). After removing the discs from the moulds they were stored in water at 37°C for three hours. Some of discs were then washed with ethanol and put to dry in vacuum while the rest were stored in falcon tubes containing five ml of distilled water each. After seven days the discs were removed from the water, washed with ethanol, and put in vacuum. A bulk reference was obtained by grinding down one of the discs using a SiC grinding paper until the original surface had been completely removed.

The samples were analyzed using both a scanning electron microscope (SEM) (LEO 1550, Carl Zeiss, Germany), in order to investigate the surface topography, as well as an X-ray photoelectron spectroscope (XPS, Physical Electronics Quantum 2000, Al Kα X-ray source), to analyze the surface composition of the seven day sample and the composition of the bulk reference sample.

RESULTS:

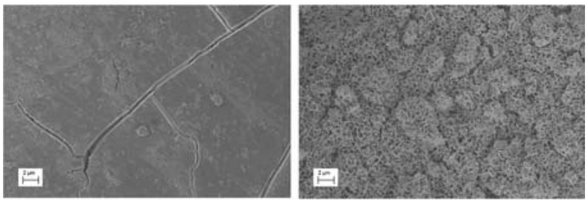

Fig. 1: SEM images the surface of ceramic samples after 0 days (left) and seven days (right) in water.

After seven days storage the surface show a flake-like structure, whereas the original surface did not show signs of any specific structure (*Fig1*). The elements analyzed with XPS were oxygen, calcium, phosphorus, aluminum, silicon, strontium, and fluorine. Results presented in *Table1* show that calcium and phosphorus have accumulated on the surface after the seven days water storage.

Table 1. XPS results showing atomic % of calcium and phosphorus on the surface of the cement stored in water for seven days and the grinded bulk reference.

	Calcium		Phosphorus	
Bulk	9.48	±0.49	0.04	±0.08
7 days in water	16.30	±0.92	3.39	±1.47

DISCUSSION & CONCLUSIONS: The XPS results show that a calcium phosphate coating has been formed on the surface during water storage. The morphology of this coating gives a good indication that it is hydroxyapatite, the mineral phase of bone. Apatite formation is possible since the cement contains all building blocks needed via its powder composition. In addition, the surface charge after hardening provides a good site for calcium phosphate nucleation and growth.

REFERENCES: [1] Lööf J. et al., Dental Materials, 2008, 24, 653–659

ACKNOWLEDGEMENTS: Doxa AB is gratefully acknowledged for financial support.

Electrospun polyurethane-based elastomer fibers for biomedical applications

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INTRODUCTION: Electrospun polymer fibers have emerged as a promising candidate for future tissue engineering and biomedical in-vivo and in-vitro appliances (1,2). Recent studies have argued that polyurethane in electrospun form have potential applications within the area of regenerative medicine employed for wound dressing, cell alignment, annalus fibrous tissue engineering, promoting endothelial proliferation, high performance filters and scaffolding promoting neuronal differentiation of human embryonic stem cells (1,2). Even though PU is a comparably well studied biomaterial that have been readily applied in biomedical applications, the complex surface interactions between electrospun PU fibers and cells are not well understood.

Oxygen plasma surface modification (PSM) is a versatile material processing technique that has been shown to selectively modify surface properties such as wettability, adhesion and biocompatibility of polymer surfaces, and can improve surface properties for cell attachment on polyurethane membranes.

METHODS: In this paper we study the interactions of native and oxygen plasma treated electrospun polyurethane with water and red blood cells. Varying plasma exposure time fiber hydrophilicity is assessed through static and advancing contact angle measurements, surface chemistry is determined with x-ray photon spectroscopy and morphological degradation is imaged with SEM.

RESULTS: Red blood cells are used as model cells and interactions with fibers are investigated focusing on cell membrane shape-changes observed live with light microscopy (Fig.1a), laser confocal microscopy (Fig.1b) and fixed in SEM (Fig.1c). Retained cell function when brought into contact with the fiber surface is appraised by quantifying exposed calcium and phosphatydilserine level.

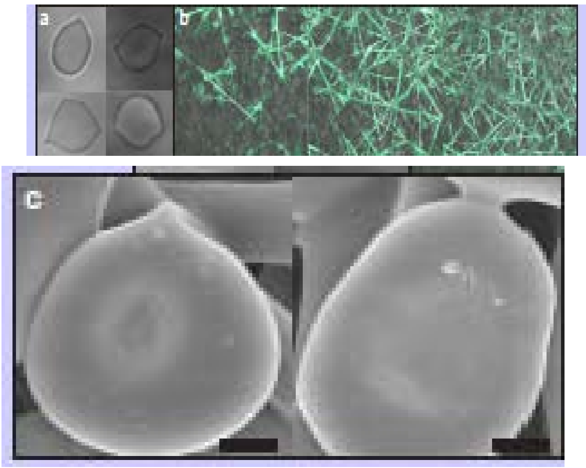

Fig. 1: Images of red blood cells brought in contact with electrospun polyurethane-based network

DISCUSSION & CONCLUSIONS: The oxygen plasma treatment is found to be a fast and feasible method to modify physicochemical surface properties of electrospun polyurethane fibers. By increasing the amount of surface oxygen bound to peroxide groups the hydrophilicity of electrospun network is increased dramatically. Utilizing long exposure times does not cause any critical change to composition or wetting behavior but instead acts to alter the morphology of the networks as well the roughness of single fibers.

Red blood cell function appears to be retained in fiber contact. Protrusions resembling point forces are observed to certain extent and cells are found to penetrate into the network. No crucial difference in RBC response was detected between na- tive and oxygen treated electrospun polyurethane fibers.

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Bioactive glass/poly(L-lactide-co-glycolide) composite scaffolds for bone tissue engineering

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INTRODUCTION: Poly(L-lactide-co-glycolide) (PLGA) has been widely used for bone tissue engineering, thanks to its biocompatibility, controllable biodegradability, and good processability. However, in its native form, e.g. without any particular modification, it does not trigger a required biological response of osteogenic cells [1]. Bioactive glasses (BG) are very promising materials able to support adhesion, growth and osteogenic differentiation of cells *in vitro* and to form a tight chemical bond with living bone tissue *in vivo* [2]. In this study two types of sol-gel derived BG were used to produce PLGA-based composite scaffolds with the aim to assess their potential as a support for bone cells in tissue engineering applications.

METHODS: PLGA (85:15, Mn= 100 kDa, d=2.1) and two types of BG, obtained by sol-gel method differing in chemical composition: molar ratio of SiO_2:CaO:P_2O_5 equal to 40:54:6 (A2 – high lime BG) and 80:16:4 (S2 – high silica BG), respectively; grain size <2 µm; were used to manufacture composites containing 10% volume fraction of BG. The composite PLGA-BG scaffolds were produced by solvent casting/salt particulate leaching method. Susceptibility to hydrolytic degradation was evaluated by soaking the scaffolds in PBS for 12 weeks. Bioactivity of the scaffolds defined as the ability to form amorphous calcium phosphates from SBF solution was tested according to Kokubo *et al.* method [3]. The scaffolds were studied by SEM and EDX and their porosity was estimated. Drop shape analysis system (DSA) was also used to evaluate water contact angle and water penetration within the scaffolds. After H_2O_2-plasma sterilization the osteoblast-like MG63 cells were cultured on the scaffolds for different periods of time; morphology, adhesion, proliferation and differentiation of the cells were evaluated.

RESULTS: PLGA-BG scaffolds with 91% porosity were produced. The scaffolds showed interconnected tortuous pores with diameters in the range of 400-600 µm (Fig.1a). SEM/ EDX show that BG particles were homogenously distributed within PLGA matrix (Fig.1b,c). PLGA-BG scaffolds were more hydrophilic than PLGA scaffolds; the water penetration time within PLGA-BG scaffolds was shorter than for reference PLGA scaffolds. Composite scaffolds produced of A2-BG enhanced deposition of calcium phosphate from SBF; this was not observed for reference PLGA and S2-BG scaffolds. Hydrolytic degradation studies show that after 12 weeks in PBS the decrease of mass of PLGA, PLGA/S2 and PLGA/A2 scaffolds was 1wt%, 5.5wt%, and 10.0 wt%, respectively. *In vitro* results show that PLGA-BG scaffolds supported osteoblast-like cell adhesion, proliferation and differentiation.

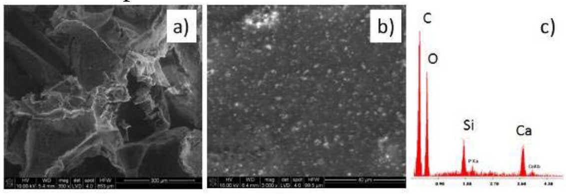

Fig. 1: SEM (a,b) and EDX (c) of PLGA/A2-BG

DISCUSSION & CONCLUSIONS: Incorporation of sol-gel derived BG into PLGA matrix improves the bioactivity, hydrophilicity and influence their degradation time. The resulting composite scaffolds are promising for bone applications.

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Nanostructured Coatings on Implant Surfaces

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INTRODUCTION: The aim of this study was to evaluate the *in vivo* effects of three nanostructured calcium phosphate (CaP) coatings on relatively smooth titanium implant surfaces.

METHODS: CaP nanoparticles having different size and morphology were coated onto implants using an immersion technique followed by a short heat treatment. Uncoated implants were used as control. After topographical and chemical characterizations, implants were randomly inserted into the tibia of twelve rabbits for removal torque (RTQ) testing. Furthermore, to confirm the biological reaction, implants were placed in the bilateral femurs of three rabbits. These were used for histology.

RESULTS: The topographical characterization showed that each surface had different nanostructural characteristics and X-ray photon spectroscopy showed various Ca/P ratios: Surface A 2.0, Surface B 1.5, Surface C 1.2. Also, the control and test groups had different nanotopographies.

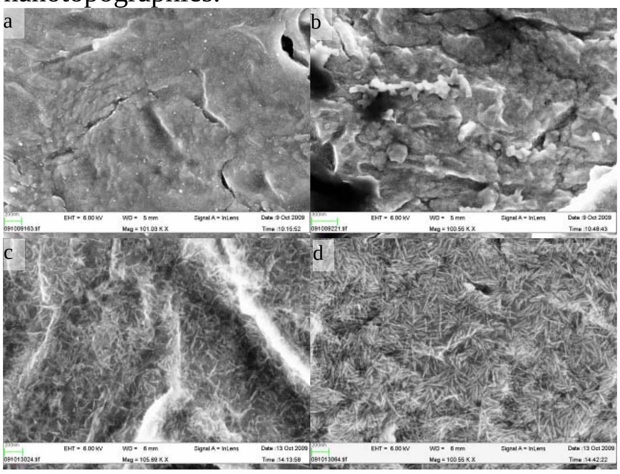

Fig. 1. SEM pictures of a) Control, b) Surface A, c) Surface B and d) Surface C.

The RTQ tests showed significantly higher values in two test groups (Surface A and Surface C), ($p<0.05$). The histological observation showed that new bone formation was successfully achieved in all the groups, without noticeable adverse effects from the CaP coating for all the test surfaces. In higher magnification, it was shown that the majority of the bone tissue in close relation to the implants was newly formed bone with large osteocytes.

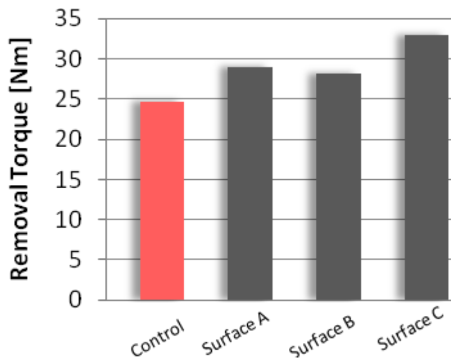

Fig. 2. RTQ test for control, Surface A-C.

DISCUSSION & CONCLUSIONS: From the topographical characterization, the CaP coatings have altered the nanotopography of the implant surfaces, without changing the microroughness. The biomechanical tests showed that both Surface A and Surface C had significantly higher RTQ values than the uncoated implant surface. This shows that the application of nanostructured CaP can improve the biomechanical bonding between implant surface and bone tissue. However, the results of this study indicate that the three different CaP-coated surfaces possess similar nanotopographies, which can enhance the osseointegration and might have influenced the positive clinical effect.

ACKNOWLEDGEMENTS: The current study was supported by the grants from the Swedish Research Council, the Hjalmar Svensson Research Foundation, Örebro University U-2000 and the Knowledge Foundation (KK stiftelsen, Biofilms-research centre for biointerfaces).

Electron Tomography: A tool for the study of nano-osseointegration in 3D

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INTRODUCTION: Understanding the interfacial structure between implant materials and bone is crucial for implant stability. The ossointegration of biointerfaces has long been imaged in two dimensions with light, x-rays and electrons. With the advances in modern day transmission electron microscopy, high-resolution visualization and analysis of three-dimensional structures is possible. We present the application of Z-contrast electron tomography to the determination of osseointegration between bone and hydroxyapatite or titanium implants on the nano-level.

METHODS: Prototype porous hydroxyapatite (HA) scaffolds [1], or dental implants (BioHelix™) not needed for clinical reconstruction, were retrieved from human bone with surrounding tissue and processed by fixation, dehydration and resin embedding. A dual-beam focused ion beam (FIB) microscope with in-situ lift out method was used for sample preparation for transmission electron microscopy. Tomographic series were collected on a Titan 80-300 transmission electron microscope (FEI Company, Eindhoven, The Netherlands) operated at an acceleration voltage of 300kV using a high-angle annular dark-field detector (HAADF). Images of the bone-implant interfaces were acquired in increments of 2°, up to tilt angles of ± 60°, and 1°, for further angles up to ± 75°.

RESULTS: Single-axis tomographic tilt-series were collected over the bone-implant interfaces and using back projection, with a simultaneous iterative reconstruction technique, three-dimensional reconstructions with nanometre resolution were created. The reconstructed 3D volume of the hydroxyapatite bone interface clearly reveals the distinct orientation of HA particles in the fibrous bone structure and in the dense interfacial apatite layers, Figure 1 [2]. Electron tomographic reconstructions over the titanium implant interface show the nano integration between surface titanium oxide layers and precipitated HA with bone, as shown in Figure 2.

Fig. 1: Orientation of HA crystallites in bone and precipitated on the surface of a bulk implant.

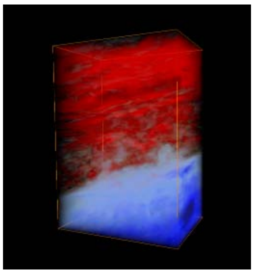

Fig. 2: 3D reconstruction of titanium (blue) – bone (red) interface.

DISCUSSION & CONCLUSIONS: We have demonstrated the ability to produce reconstructed volumes of the nanometre scaled regions between both titanium and hydroxyapatite implants and human bone. Viewing these structures in three dimensions enabled us to observe the nanometer differences in morphology of the implant surface. Insight into the 3D structure of a vast number of biointerfaces is possible with electron tomography and may transform the approach to device design and the study of osseointegration.

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Pore spanning lipid bilayers on mesoporous silica having varying pore size

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INTRODUCTION: Synthetic lipid bilayers have similar properties as the living cell membrane, which can be utilized in the development of biomimicry devices, such as biosensors. One promising design for such a sensor is to use the lipid bilayer as a host for transmembrane proteins, which can be utilized as sensing elements.[1] In such an application it is essential to create a device that provides mechanical robustness to the lipid bilayer and simultaneously gives rise to a suitable environment for the transmembrane proteins. This is suggested to be provided by having mesoporous supported lipid bilayers.[2] The porous structure would result in a stable support due to the pore walls and at the same time provide adequate environments both below and above the pore spanning bilayer, which is essential for obtaining functional proteins. The properties of mesoporous materials, such as, the pore size, pore geometry and surface chemistry can be tailored, which allows us to precisely design the support according to our needs.

METHODS: Formation of lipid bilayers on mesoporous silica having pore sizes of 2, 4 and 6 nm was investigated using quartz crystal microbalance with dissipation monitoring (QCM-D), fluorescent recovery after photobleaching (FRAP) and atomic force microscopy (AFM). Mesoporous silica was synthesized according to the evaporation induced self-assembly process using P123,
BrijS10 and CTAB as templating molecules.

RESULTS: Lipid bilayer formation was observed on all surfaces regardless of porosity. QCM-D results indicated that the pore size had an impact on the bilayer formation kinetics; with the most rapid formation on the surface having the smallest pores, as is shown in Fig. 1.

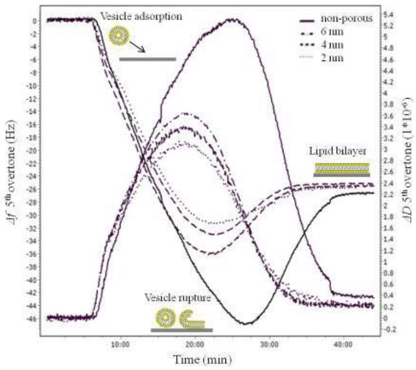

Fig. 1. QCM-D results showing formation of lipid bilayers on all surfaces with a more rapid formation of the bilayer on the surface having the smallest pores.

The lateral diffusion of the lipids was observed using FRAP and was found to be lowest on the surfaces having the smallest pores and increased with increasing pore size. Furthermore, results obtained using AFM concluded that vesicles were more prone to rupture on mesoporous silica than on non-porous silica. Contact angle measurements showed that the surface having the smallest pores was the most hydrophilic surface and that the hydophilicity decreased with increasing pore size.

DISCUSSION & CONCLUSIONS: The results showed that bilayers were successfully formed on mesoporous silica, however, the pore size was observed to have an impact on the bilayer formation. This impact is suggested to be due to the hydrophilicity of the surfaces, a more hydrophilic surface resulted in a more rapid formation of bilayers. Mesoporous silica is considered to be a promising support for lipid bilayers in biosensing devices.

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Surface morphology of modified titanium and titanium-zriconium surfaces after cathodic polarization

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INTRODUCTION: Etching of titanium in acids can induce the formation of TiH_2 on the samples surface due to a reaction of liberated H^+ ions with the metal surface [4]. As the hydride modified surface was shown to be favourable for implant applications approaches have been made to form thicker layers by cathodic polarization [2]. As this technique involves etching processes a further modification of the sample surface takes place. This effect will be evaluated with respect to crucial topographical surface parameters on titanium and titanium zirconium implants.

METHODS: The samples used in this study were grade 4 titanium SLActive (Ti) and titanium-zirconium SLActive (TiZr) coins with a diameter of 4.39 mm and a height of 2.0 mm (Straumann, Basel, Switzerland). The polarization was executed at room temperature in 2 molar acetate buffer.

For the topography analysis a Plu 2300 blue light profilometer was used (Sensofar, Terrassa, Spain). Every sample was analyzed on four different randomized spots. Each spot corresponded to a surface area of 253x190 µm.

The paramters analyzed were chosen according to Lamolle et al. [3].

RESULTS: Comparing titanium and titanium zirconium, Sa values were found to be higher in general for TiZr, ranging up to 3 µm. Deviations of the surface parameters were more noticeable for Ti with respect to time and current density of treatment. Changes in microstructure were observable for titanium samples from the profilometer measurements and correlation study.

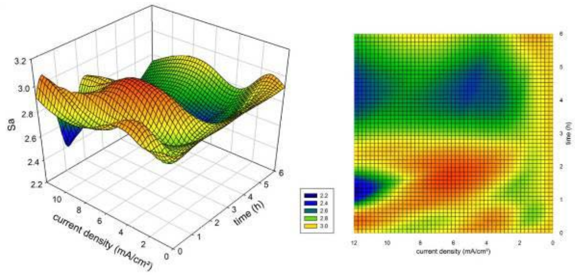

Fig. 1: Sa value deviation in correlation to treatment time and applied current density on TiZr.

DISCUSSION & CONCLUSIONS: The results of the present study indicate a connection between higher mechanical properties[1] and chemical stability of the TiZr alloy. The surface structure of titanium was not found to be altered significantly

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Impact of Nanostructure on Bacterial Adhesion and EPS Production

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INTRODUCTION: Biomaterial-related infections can result in the failure of implant function and can cause serious suffering for the patient. In a previous study we found that the nanostructure of a commercial anti-microbial biomaterial coating affected early protein adsorption and blood compatibility[1]. Here we investigate the importance of the nanotopography of a model biomaterial surface for the initial adhesion of bacteria. By the use of Quartz Crystal Microbalance (QCM-D)[2], fluorescence microscopy and Scanning Electron Microscopy (SEM) we have studied the adhesion and production of EPS (extracellular polymeric substances) by *S. epidermidis* on smooth and nanostructured gold substrates.

METHODS: Nanostructured substrates were created by immobilizing gold nanoparticles in the range of 10-20 nm on smooth (Rms<1nm) gold surfaces using a SAM of cysteamine. All surfaces were washed with basic piranha to yield surfaces with different topography but with identical chemistry. Starved *S. epidermidis* were incubated for 5h at room temperature on smooth or nanostructured surfaces in standard petri dishes or in QCM-D. The cells were then stained with a EPS specific fluorescent dye (Film Tracer SYPRO Ruby biofilm matrix stain, Invitrogen) or Osmium tetroxide.

RESULTS: The production of EPS and the number of attached bacteria was markedly impeded on the nanostructured surfaces after 5 h of incubation as seen in fig. 1.

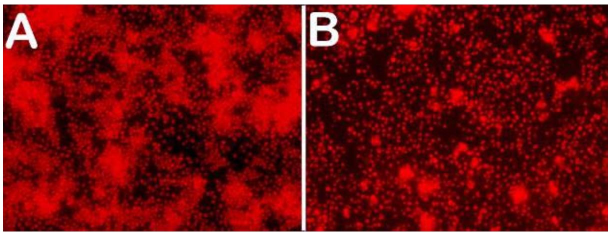

Fig.1. Fluorescence microscopy of S.epidermidis stained with a biofilm-specific dye. A: Smooth surface. B: Nanostructured surface.

The morphology of the attached bacteria was found to be affected by the nanotopography; Cells attached to the smooth surfaces generally showed a more flattened appearance and looked more firmly attached to the surface. On the nanostructured surfaces the cells did not seem to interact with the surfaces to the same extent, and the shape of the cells appeared to be more rounded (see fig.2).

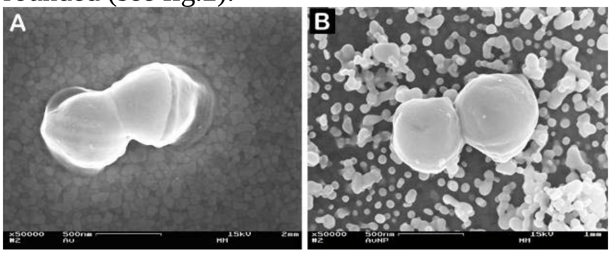

Fig.2. SEM depiction of attached bacteria on a flat gold surface (A), and on a nanostructured gold surface (B).

Initial results from measurements in QCM-D indicated that the cells were more firmly attached to the smooth surfaces (fig.3).

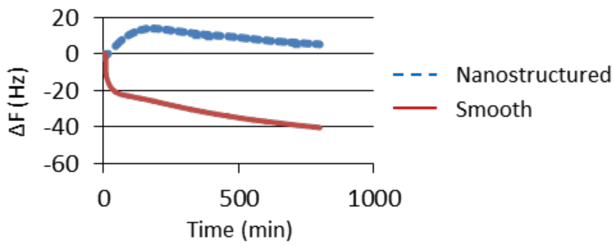

Fig.3. Bacterial adhesion studied in QCM-D for 13h on smooth and nanostructured surfaces. Note the difference in frequency shift.

CONCLUSIONS: Based on our findings we conclude that the nanotopography of a surface affects the initial bacterial adhesion and production of EPS.

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Surface morphology of modified titanium and titanium-zirconium alloy surfaces after anodic polarization

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INTRODUCTION: Titanium, zirconium and their alloys are known for their excellent biocompatibility and mechanical properties. Various studies showed the importance of the surface's chemistry and morphology to implant integration into bone. Modification of the TiO_2 layer, sandblasting or acid etching were some of the approaches that showed promising results in earlier studies [1; 4]. While cathodic polarization of titanium showed increased layers of titanium hydride, anodic polarization showed creation of hydroxide groups on the surface [2; 3]. This study aimed at identifying possible surface changes caused by the anodic polarization process.

METHODS: This study used coins made from commercially pure titanium grade 4 commercially known as SLActive® (Ti) and a titanium-zirconium alloy (TiZr) commercially known as Roxolid® (Straumann, Basel, Switzerland). All samples were provided with SLActive® surfaces (Straumann, Basel, Switzerland). Polarization was done in NaOH solution at room temperature for 30 and 60 minutes at 0.5, 1 and 3 mA/cm^2.

A PLµ 2300 (Sensofar-Tech S.L., Terrassa, Spain) blue light profilometer and interferometer using a 50x EPI (Nikon, Tokyo, Japan) confocal objective was used for assessing the surface topography.

A Hitachi Tabletop Microscope TM-1000 (Hitachi Europe GmbH, Krefeld, Germany) was used for SEM imaging.

RESULTS: Assessment of the surface roughness (S_a) for Ti and TiZr revealed that surface roughness generally increased after anodic polarization. When compared to the untreated (1.68µm) anodized Ti samples showed a maximum roughness of 2.17µm (30min, 3mA/cm^2). For TiZr untreated samples revealed a roughness of 1.86µm while anodized samples showed values up to 2.04µm (30min, 0.5mA/cm^2). Statistical analysis confirmed significant differences to the untreated sample for both materials.

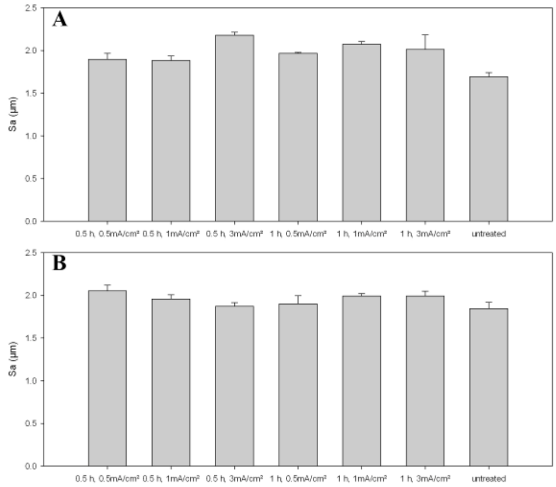

Fig. 1: Surface Roughness (S_a) for titanium (A) and titanium-zirconium (B).

DISCUSSION & CONCLUSIONS: The observed changes in surface micro-morphology suggest an alteration of the surface of some kind. It is highly likely that the surface chemistry experiences changes and builds up hydroxide deposits during the process.

Whether the significant differences in surface morphology has a influence on the surface's in-vitro or in-vivo performance , is yet to be confirmed.

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Hybrid nanoparticle arrays for measuring the interaction between cell adhesion ligands and macromolecules using SPR.

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INTRODUCTION: By investigating the number and morphology of primary human endothelial cells grown on arrays of negatively charged dendrimers and linear macromolecules of similar size, it was seen that the dendrimer potentiated cell-growth to a larger extent than the linear counterpart [1]. As we anticipate a non-specific adhesion mechanism the results suggests that the dendrimer-functionalized surface adsorbs and makes bioavailable a greater amount of cell interacting ligands then the linear counterpart when applied at similar surface coverage ratios. As an example of such ligand we chose to characterize the binding of endothelial cell growth factor (ECGF) and albumin directly onto macromolecular arrays prepared on commercial gold substrates for surface plasmon resonance (SPR) analysis.

METHODS: To arrange polymeric entities at the nanometer scale, self-assembled, short-range ordered Au-nanoparticle (NP) arrays were used as a versatile template [1-2]. By varying the ionic strength upon assembly particle coverage ratios between 10 to 30% was obtained as determined using SEM, Figure 1. Recombinant human ECGF (MW 17kDa) was injected in series of concentrations ranging from 5 to 200 nM onto the array-surfaces using a Biacore 2000 system.

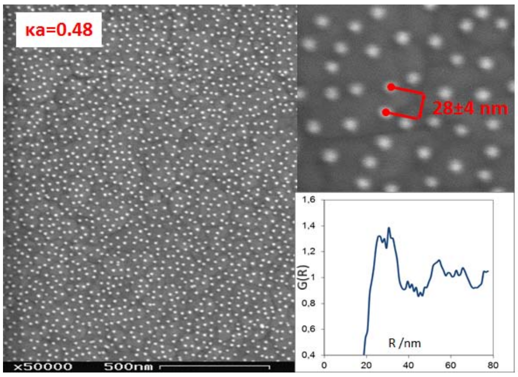

Figure 1. The interparticle distance as determined with SEM at deposition condition, κa = 0.48, together with the radial distribution. The particle coverage was determined to 10%.

RESULTS: Injection of ECGF gave rise to large binding both on dendrimer and linear polymer modified arrays, Figure 2. It is also clear that ECGF binding to linear polymer arrays saturates and dissociates more quickly compared to the dendrimer loaded arrays. Albumin displayed only weak adsorption when injected onto the dendrimer coated array.

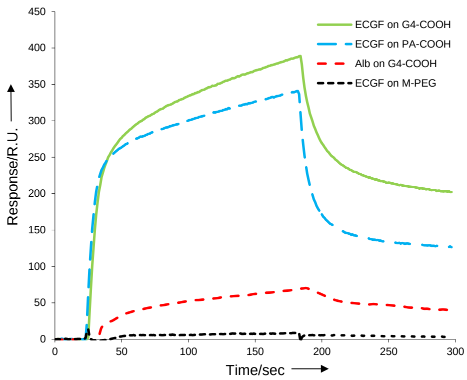

Figure 2. Representative SPR sensorgrams for the ECGF binding to NPs modified with G4-COOH dendrimer (solid line) and PA-COOH (long dashed line). Low amounts of Albumin binding were observed on the G4-COOH modified NPs (short dashed line). The majority of the ECGF binding is mediated via the NP macromolecular hybrid complex based on the low amounts of ECGF found on the M-PEG control surface (dotted line).

DISCUSSION & CONCLUSIONS: ECGF is a very strong endothelial cell mitogen. We suggest that negatively charged synthetic polymers may adsorb ECGF preferentially from a solution of different proteins and by this control the cell adhesion process. In this study differences in macromolecular architecture had a profound effect on the binding of ECGF and a higher loading capacity was reached for dendrimer coated particles. We expect the high charge density and the branched shape of the dendrimer to be responsible for the differences in ECGF binding.

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[2]Lundgren A. et al. Nanoletters 2008, 8, 3989-3992

Preparation of peptide-functionalized Au nanodot and lipid bilayer surfaces for studying cell focal adhesion formation

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INTRODUCTION: Cell-substrate interactions are important cues for stem cell differentiation and cellular behavior. The research groups of B Geiger and J Spatz studied previously in great detail focal adhesion (FA) formation on cRGD-peptide functionalized gold nano dots. By varying the interparticle distance with nanometer precision, they proposed the optimal distance for FA formation to be >72nm.[1]
This study builds upon their results by combining immobilized ligands on gold nano dot surfaces with mobile ligands in a supported lipid bilayer, (Figure 1). Such a system offers the possibility to provide signaling cues to the cells via the bilayer, while ligands bound to Au nanodots can control the cell adhesion and spreading.

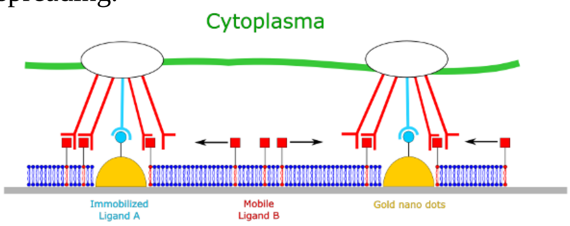

Fig. 1: Schematic of surface modification with ligands to control cell adhesion and cell stimulation using a substrate with immobilized ligand A on the gold nano dots and mobile ligand B in the bilayer, respectively.

METHODS: The whole process of bilayer formation by vesicle fusion and functionalization with ligands was integrated on a microfluidic platform. Bilayer formation around Au nanodots was studied by fluorescence microscopy and quartz crystal microbalance with dissipation monitoring (QCM-D) measurements.

Cell adhesion was evaluated by total internal reflection microscopy (TIRFM) and bilayer mobility by FRAP measurements. Three different bilayer functionalizations were investigated: charged lipid head groups, maleimide-thiol coupled cRGD peptide and biotin-streptavidin coupled of cRGD peptide.

RESULTS: The data suggest that functionalization of the gold nano dots prior to bilayer formation is necessary to avoid adsorption of intact vesicles on the gold. These results need to be further evaluated by microscopy and quartz crystal microbalance with dissipation monitoring (QCM-D) measurements.

Functional coupling of cRGD peptide via biotin-streptavidin chemistry was monitored by QCM-D. It was possible to maintain bilayer mobility by using a maximum of 0.1% biotinylated lipids in a Palmitoyl-oleoyl-phosphatidyl-choline (POPC) matrix. The next step will be to culture cells on the functionalizaed lipid bilayers, in order to investigate FA formation on a laterally mobile substrate.

DISCUSSION & CONCLUSIONS: The advantage of this approach is that the cells can freely arrange the mobile ligand in the bilayer, allowing fast evaluation of optimal spacing in multi-ligand systems. This information could then be used to design appropriate, large scale, cell scaffolds with optimized properties.
This is work in progress and more experiments need to be carried out to confirm initial results and develop robust protocols to evaluate cell adhesion and differentiation on these versatile substrates.

REFERENCES: [1] Cavalcanti-Adam et al., Biophys J. 2007 Apr; 92: 2964–2974.

ACKNOWLEDGEMENTS: The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° NMP3-SL-2009-229294 NanoCARD.

No effect of systemic strontium on early implant fixation in rat tibia

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INTRODUCTION: Strontium (Sr) containing drugs increase bone density in osteoporotic patients, although possible improvements in the fixation of bone implants are not well studied. Other osteoporosis drugs are, however, known to improve the fixation of intraosseous implants, and we therefore compared the effect of systemic strontium treatment to systemic alendronate (BP) treatment in an implant model that was previously used for the analysis of drug effects.

METHODS: Stainless steel and PMMA screws were inserted into tibia (left/right) of male Sprague-Dawley rats for 4 weeks. The animals were fed pellets with or without addition of strontium ranelate (800mg/kg/day). The positive control group received alendronate subcutaneously. All animals were sacrificed after the implantation period and the pullout strength, microCT and histological analysis were assessed on the implanted tibias.

RESULTS: No effect in pullout force was observed for the strontium ranelate systemic treatment group (p = 1,000; 95% CI: decrease by -20 to increase by 21%) compared to untreated control. However, the systemic treatment with alendronate showed a significant effect compared to strontium ranelate and the untreated control (p = 0,000; 95% CI: increased 27-67% / p = 0,000; 95% CI: increased 28-67%).

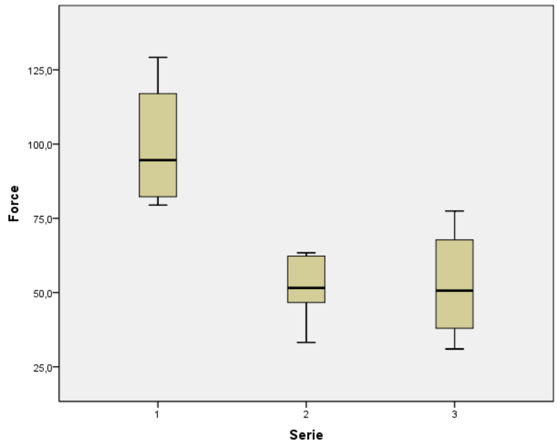

Fig. 1: Screw pull out force after 4 weeks in rat tibia for systemic bisphosphonate treatment (1), systemic strontium ranelate treatment (2) and for untreated control (3).

DISCUSSION & CONCLUSIONS: These results contrast to previous studies using the same model, where systemic parathyroid hormone or local bisphosphonate each doubled the pullout force after 4 weeks of implantation. Strontium has a known reinforcing effect on bone, but it seems this effect is weak in conjunction with bone repair or early implant fixation. We conclude that other treatments may be more efficient for the improvement of early implant fixation.

Regulation of complement activation at a surface by capturing factor H

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* Authors contributed equally to this work.

INTRODUCTION: Implantation or extracorporeal use of biomaterials, as well as transplantation of cell clusters is hampered by adverse reactions related to the activation of innate immunity and proinflammatory pathways. The complement cascade plays a vital role in these events by attracting and activating inflammatory cells, and is thus a significant target for protection [1]. Self-cells exposed in the blood stream are protected from complement activation via membrane bound and soluble regulators. Factor H, which is the most abundant soluble regulator in plasma is recruited to cells via interaction with e.g. glycosaminoglycans [2]. Here, we take this concept of capturing soluble regulators to a non-self surface. The aim of this project was to inhibit complement activation by attracting factor H to a surface, making it self-regulatory, using the patient's own regulators, analogous what happens on our own cells.

METHODS: A peptide (5C6) with affinity to the middle region of factor H [3] or a scrambled control peptide was immobilized onto a polystyrene surface using biotin/avidin chemistry, or introduced to the membrane of rabbit erythrocytes and porcine endothelial cells via a hydrophobic lipid tail. Recruitment of factor H to the surface and cells was verified with ELISA and confocal microscopy respectively. Hirudin anticoagulated human plasma or whole blood was incubated on the peptide-coated material and complement activation was examined by measuring soluble (C3a, sC5b-9) and surface (C3-fragments) activation markers. To investigate the effect of the 5C6-peptide on cells, this peptide or the scrambled variant were introduced onto rabbit erythrocytes and porcine endothelial cells. Complement activation was examined by measuring erythrocyte lysis and soluble (C3a, sC5b-9) activation markers respectively.

RESULTS: Factor H from the fluid phase of plasma and whole blood was specifically enriched and bound to materials and cells having the 5C6-peptid. Compared to the control-scrambled peptide, materials exposing the 5C6-peptide showed lower levels of complement activation markers in the solution and on the surface. Cells having membrane bound 5C6-peptide were to a significant degree protected from complement attack; lysis of rabbit erythrocytes in human serum was attenuated and porcine endothelial cells induced lower levels of C3a and sC5b-9 when exposed to human whole blood.

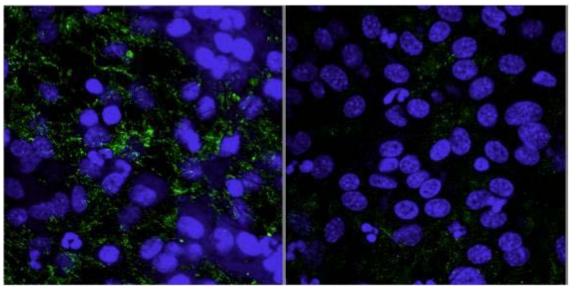

Fig. 1: Binding of factor H to porcine endothelial cells with 5C6-peptide (left) and scrambled control-peptide (right). Factor H detected with FITC-labelled antibody (green) and nuclei staining in blue.

DISCUSSION & CONCLUSIONS: The aim of this project was to inhibit complement activation at a non-self surface by capturing factor H to a model biomaterial or to cells. Our data show that recruitment of factor H from the fluid phase to the surface of a material or cells protect it from complement attack. Both materials and cells exposing the factor H binding peptide showed decreased levels of complement activation markers. This peptide could be a tool for protecting surfaces from complement activation upon implantation of biomaterials or transplantation of cell clusters, decreasing inflammation and improving clinical outcome.

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Unique IPN technology prevents bacterial resistance

Robert Lessèl
Chemmpilots a/s,

INTRODUCTION: Newer Danish licensable impregnation technology use supercritical Carbon dioxide (sCO_2) to produce hybrid materials with self-sustainable, e.g. antibacterial, surface maintained by a nano interpenetrating network (IPN). The IPN-impregnation technology is unique for manufacture of long-term implantable silicone based devices, such as catheters, and other types of controlled release devices or medical devices where special surface properties is requested.

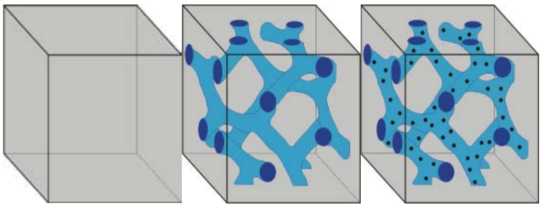

Fig. 1: IPN silicones - i*mparting sustained drug delivery to existing silicone based medical devices. The three blocks represent the process steps in the IPN impregnation process going from virgin silicone (left) to impregnated IPN copolymer system (middle; IPN=blue) and to API loaded IPN network (right).*

METHODS: Supercritical carbon dioxide (sCO_2) is used as a "sophisticated" solvent in impregnation processes for manufacture of the robust IPN based polymeric systems. This poster presentation gives a general introduction to sCO_2 processing (impregnation, polymerisation, loading of API etc.), equipment and possibilities.

RESULTS: Chempilots is part of a DNATF supported project starting in April this year with special focus on development of robust impregnation process for silicones with the aim of preventing biofilm formation and medical device induced infections and related complications by long-term sustained release of effective, low doses of locally/ surface active and stable antimicrobial peptides (new AMP mimetics). Data form Proof-of-concept will be discussed including initial in-vitro release studies with ionic silver, sulfonamides and AMPs.

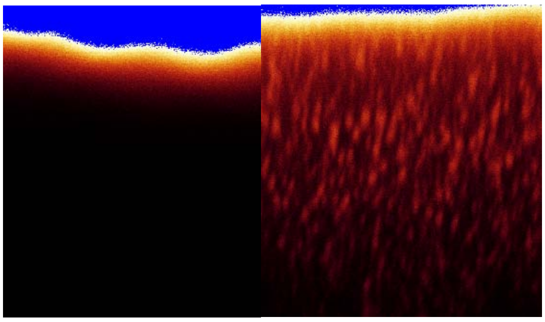

Fig.2:Laser scanning confocal microscopy showing a polyHema IPN hydrogel network within a silicone polymer;white droplets contains"contrast agent". Left: virgin silicone.

DISCUSSION & CONCLUSIONS: The IPN impregnation technology is an "Add-on" process, unique for silicone based medical devices where a hydrophilic IPN/ hydrogel is created within a silicone polymer. Initial results point at surface antibacterial effects for more than 25 days preventing formation of biofilm on catheter surfaces and related infections that normally requires long-term systemic treatment with high doses of antibiotics, the latter with risk of development of resistance towards antibiotics. Chempilots will within few months have established facilities to run the IPN impregnation process in ISO class 7 or 8 clean room for fabrication of real samples for companies from the medical device industry. It will also be possible for companies to obtain a non-exlusive licens through Chempilots for use of the IPN impregnation technology.

REFERENCES:
WO05003237 & WO2008/052568: methods of producing interpenetrating polymer networks (IPN), the IPN and use thereof etc. owned by Biomodics.

ACKNOWLEDGEMENTS: The work is part of Proof-of-concept for a DNATF (Danish National Advanced Technology Foundation) project cf. j. no. 041-2010-3.

Højteknologifonden

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Wettability of porous glass-fibre reinforced composite implant material with blood

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INTRODUCTION: Glass-fibre reinforced composites (FRCs) are under current investigation to serve as durable bone implant materials for load bearing orthopaedic applications [1] and in head and neck surgery. [2]

In previous *in vivo* experiments the transport of body fluids by capillary penetration into porous implants made of FRC laminate structures was observed. The aim of this *in vitro* study was to enhance the blood flow into the pores. This may positively influence the level of bone ingrowth.

METHODS: The biocompatible FRC was made of non-woven E-glass-fibre tissues (semi random fibre orientation) impregnated with a non-resorbable, photopolymerisable bifunctional polymer resin with equal portions of bisphenyl-A-glycidyl dimethacrylate (BisGMA) and triethyleneglycol dimethacrylate (TEGDMA). The mass percentages of resin in the FRC were controlled by different levels of compression and air-blow.

Firstly, five groups of FRC with a total porosity in the range of 10-70 vol% were prepared, more than 90 vol% of which being functional (open pores), and the rest closed. The pore sizes were above 100 µm. Wettability characteristics were assessed via contact angle measurements and a dipping test using manufactured liquids (simulated blood) as well as human blood.

One FRC group (49-55 vol%) was selected for the second part of the study: laminate structures (Fig. 1a) containing bioactive glass granules (BG) of three fractions were prepared: <50 µm (A); 100-250 µm (B) or 500-800 µm (C). The penetration depth of the test liquids was monitored.

RESULTS: The wettability of the FRC surface (increase of water contact angles from 88.3 ± 6.6° to 126.6 ± 9.8°) and the liquid penetration into the structure increased as a function of the degree of interconnected microporosity of the FRC. The penetration depths were highest in the predominant fibre direction. A change of hydrophobicity of the artificial blood had a higher impact on the penetration than the variations in the test liquid viscosity.

The penetration into FRC-BG laminates was deepest for fraction B. This corresponds to a gap width to BG fraction ratio of 3:1 to 4:1 (Fig. 1b).

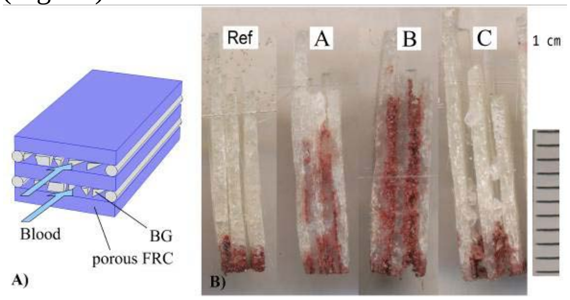

Fig. 1: Structure of the FRC-BAG laminates (A) and cross sectionional images taken after 5 s dipping in human blood (B).

DISCUSSION & CONCLUSIONS: The observed capillary penetration of the simulated and human blood into the porous FRC indicates a potential positive effect on the bone ingrowth into the implant. By adding hydrophilic BAG granules to the composite this effect can be enhanced. The fraction size of BG also affects the influx of blood.

REFERENCES: [1]Zhao et al., Med Eng Phys. 2009 31(4):461-9. [2]Tuusa et al., J Biomed Mater Res B Appl Biomater. 2008 84(2):510-9.

ACKNOWLEDGEMENTS: S.N. and A.Y.-S. are supported PhD students of the Finnish National Doctoral Programme of Musculoskeletal Disorders and Biomaterials (TBDP). This study was financially supported by the Academy of Finland. The work is part of the BioCity Turku Biomaterials Research Programme.

Nanostructured noble metals on implants *in vitro* and *in vivo*

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INTRODUCTION: Surface modifications of medical implants are of interest in order to diminish potential adverse reactions, such as chronic inflammation or infection, and to improve the integration between material and tissue. Modification with nanostructured noble metals has previously been shown to reduce the risk of infection with up to 50% when applied on urinary catheters and central venous catheters [1, 2]. Major questions to address are 1) the mechanisms for noble metals in counteracting microorganisms, and 2) the results of interaction between noble metal elements for cell response and biocompatibility. The latter is of particular importance also for novel applications in the medical field. Since biocompatibility is of utmost importance and needs to be thoroughly evaluated, we have evaluated varying noble metal modifications in three different settings: in fibroblast cultures, in rat soft tissue and in rabbit bone.

METHODS: Noble metal modifications with varied ratios of gold, silver and palladium (Bactiguard AB, Sweden) were produced on silicone (PDMS) sheets that were punched into discs for use in fibroblast cultures (1, 3 days) and in a subcutaneous rat model (1, 3, 21 days). Unmodified PDMS was used as a control. For evaluation of bone response, titanium screws with and without noble metal modification were implanted in rabbit femur and tibia (6, 12 weeks).

RESULTS: The noble metal modifications affected fibroblast adhesion *in vitro* as well as inflammatory cell recruitment in soft tissue. In general, all modified surfaces showed equal or lower inflammatory response than the control. None of the tested surfaces showed signs of extended, i.e. chronic, inflammation. Interestingly, the modifications also seemed to modulate the fibrotic response, since an increasing total amount of noble metals resulted in an increasing capsule thickness. The bone response was similar for both types of titanium implants as measured by histomorphometry (bone area within threads and bone-implant contact), with only one statistical significant difference in femur after 12 weeks in favour of the control implant.

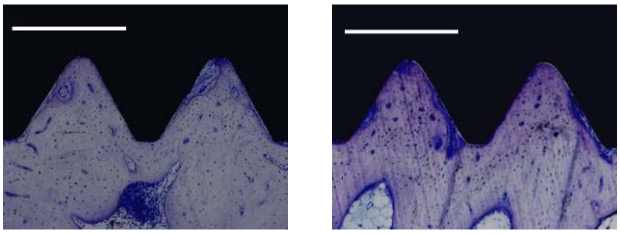

Fig. 1: Bone around noble metal modified (left) and control (right) machined titanium implants after 12 weeks in femur. Scale bar is 500 µm.

DISCUSSION & CONCLUSIONS: The results show good biocompatibility of the noble metal modifications in both soft tissues, where the inflammatory response was equal or less compared to the PDMS control, and bone, where the modified surfaces gave similar bone response as controls. In addition, altering noble metal composition on the surfaces appears to modulate cell behaviour, as indicated by differences in fibroblast adhesion, inflammatory response and fibrous encapsulation. In conclusion, we have shown that nanostructured noble metal modifications of different substrate materials are biocompatible. Provided that the previously observed inhibition of infection on catheters can be obtained also in soft tissues and bone, the present data indicate that noble metal surface modifications may have dual functions, i.e. both biocompatibility and infection resistance.

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ACKNOWLEDGEMENTS: The support from the Swedish Research Council (Grant K2009-52X-09495-22-3), BIOMATCELL VINN Excellence Center of Biomaterials and Cell Therapy, Bactiguard AB, Felix Neubergs foundation and Hjalmar Svensson foundation are gratefully acknowledged.

Antibacterial activity of superparamagnetic iron oxide nanoparticles

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INTRODUCTION: Medical devices are susceptible to microbial colonization after implantation. These infections are chronic because bacteria form a sticky slime matrix (called a biofilm) and antibiotic treatments do not resolve such infections (due to resistance). Here, the multifunctional properties of superparamagnetic iron oxide nanoparticles (or SPION) were explored for their antibacterial activity and drug delivery properties towards infection treatment via magnetic control of separating and killing bacteria.

METHODS: To produce SPION with coatings, synthesis was carried out using high temperature reflux of iron(III) acetylacetone in triethylene glycol, after which dimercaptosuccinic acid (DMSA) was added followed by silver, zinc, and iron salts. The physical properties of SPION were determined using transmission electron microscopy (TEM), vibrating sample magnetometry (VSM), X-ray diffraction (XRD), zeta potential measurements, and inductively coupled plasma atomic emission spectroscopy (ICP-AES). *Staphylococcus aureus* (#25923, ATCC) was cultured in Tryptic Soy Broth (TSB; MP Biomedical), agitated for 18 hours, and finally diluted at 1:100 when seeded onto 96-well plates for incubation (at 37 °C, 5% CO_2, humidified environment) for biofilm formation. SPION with coatings were allowed to interact for two hours prior to magnetic field exposure (4871 Gauss; K&J magnetics, Jamison, PA). Bacteria functions were then analyzed by optical density (for growth) or crystal violet (for biofilm formation) after 48 hours.

RESULTS: Results of this study showed that the as-synthesized SPION had a size of about 10 nm and saturation magnetization of 58.6 electromagnetic units per gram (emu/g). Coatings were confirmed by ICP-AES. Antibacterials were potent and it was found that silver was the most effective at killing bacteria and zinc was the most effective at decreasing biofilm formation as determined by serial dilutions (results not shown). After magnetic field application, bacteria were separated from by SPION (Fig. 1). Further nano-scale analysis confirmed that SPION coated the bacteria resulting in bacterial clustering (Fig. 2).

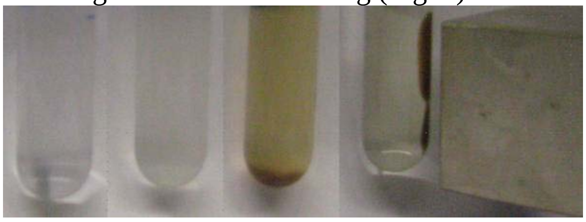

Fig. 1: Bacterial flocculation and magnetic separation can be seen in the presence of SPION. From left to right, phosphate buffered saline, bacteria, SPION and bacteria, and SPION and bacteria plus magnet.

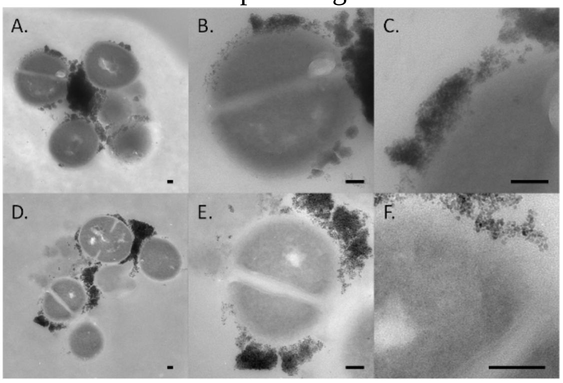

Fig. 2: Groups of bacteria interact with SPION resulting in clusters formation (A&D) mediated by SPION at the bacterial cell surface through aggregation (B&E) and surface coating (C&F). These representative images demonstrate a similar process with both metal coated SPION (zinc coated shown; A-C) or uncoated (D-F). Scale bars represent 100 nm in all images.

DISCUSSION & CONCLUSIONS: This study provided the first evidence that SPION are useful for treating infections on medical devices, or other antibiotic resistant infections. SPION alone have interesting antibacterial and anti-biofilm properties, enhanced through antimicrobial coatings, or when controlled with a magnetic field.

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Preparation and characterization of ion doped calcium phosphate spheres

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INTRODUCTION: Calcium phosphates, such as hydroxyapatite, are the most important bioceramics in bone tissue engineering because of their inherent advantages [1]. Synthesis of calcium phosphate with different forms, such as micro- and nano-particles with varied morphologies, attracts a lot attention on their applications and crystal growth. Hollow and porous spheres have recently become attractive in drug and ion delivery. Calcium phosphate could be a good candidate for such purposes. The most common methods of preparation of spherical hydroxyapatite (HA) are surfactants-conducted process [2-3]. Large HA particles could be synthesized using this method. Porous structure could be obtained, but hitherto it has been impossible to prepare hollow spheres. In this study, a self-assembled mineralization process without any surfactant has been used to prepare hollow and porous ion doped calcium phosphate spheres in micro- and nano-size. Incorporation of inorganic ions can not only deliver ions, which have pharmaceutical effects, but influence the structure of calcium phosphate spheres.

METHODS: All raw materials used were ordered from Sigma, and used without any purification. Calcium, magnesium, strontium ions were been added into phosphate buffer solution (PBS) in different concentrations. The as-prepared solutions were put into tightly covered glass bottles, and treated at 100°C. The precipitated particles were filtered and washed using ethanol and deionised water.

The morphology of the prepared particles was studied with a scanning electron microscope (Zeiss LEO 1550) and transmission electron microscopy (FEI Titan 80-300 (S) TEM). X-Ray diffraction was used to determine the crystallinity.

RESULTS: Nano- and micro-size calcium phosphate spheres were prepared using the mineralization method. The particle size ranged from 110 nm to 20 µm. The structure was solid, porous and hollow. Both magnesium and strontium ions influenced the particle size and the morphologies. All of particles formed were poorly crystallized hydroxyapatite (e.g. Fig 1 and 2). Magnesium ion affected the porous structure, and strontium induced the formation of the hollow structure (based on TEM analysis, not shown in this abstract.)

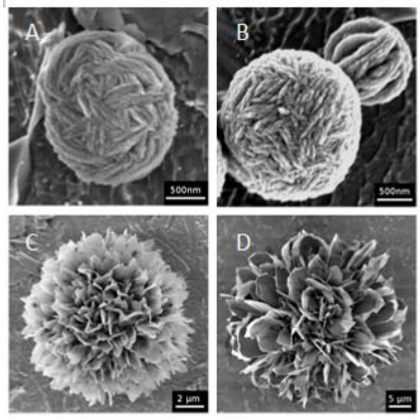

Fig. 1: Influence of magnesium and strontium on the morphology of hydroxyapatite spheres: (A)[Sr]=0.6mM, [Mg]=0.5mM, (B)[Sr]=0 mM, [Mg]=0.5mM,(C) [Sr]=0.6mM, [Mg]=0 mM,(D) [Sr]=0.6mM, [Mg]=0.5mM.

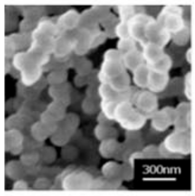

Fig.2: Morphology of hydroxyapatite nanospheres.

DISCUSSION & CONCLUSIONS: Ion doped calcium phosphate spheres with porous and hollow structures have been synthesized using the mineralization method. An aggregation-based growth of calcium phosphate could be contributed to the formation of spheres.

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Liquid crystal templating of nano-calcium phosphates

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INTRODUCTION: Liquid crystalline (LC) phases having water domains in the size range 5-10 nm can be used as templates for the formation of nanoscale calcium phosphates (CaPs).[1] In the present study, LC phases have been utilized to synthesize amorphous calcium phosphates (ACP) with different morphologies. Furthermore, a controllable aging route has been performed on ACPs for the formation of bone-like apatite.

METHODS: All chemicals were purchased from Aldrich (reagent grade). For synthesis using LC, $Ca(NO_3)_2 \cdot 4H_2O$, 85 % H_3PO_4 (with a Ca/P ratio of 1.67) were dissolved in Milli-Q H_2O. Then by mixing the salt solution with the surfactant Pluronic® L64 ($EO_{13}PO_{30}EO_{13}$) and *p*-Xylene, a reverse hexagonal (H_2) LC phase (15 wt% salt solution, 70 wt% L64 and 15 wt% p-Xylene) and a lamellar (L_α) LC phase (35 wt% salt solution, 55 wt% L64 and 10 wt% p-Xylene) were formed.[2] The LC phase was placed in an ammonia atmosphere (ammonium hydroxide, 35 wt% aq) to increase the pH, thus to initiate the reaction within the water domains. After 72 h, the reaction was stopped and the obtained material was washed repeatedly using Milli-Q H_2O and 95 % ethanol, then freeze-dried. For the aging route, as-prepared ACP powders were dispersed in Milli-Q H_2O (0.5 wt%) and aged at room temperature for different times. Then the particles were purified and dried as described above.

RESULTS: Figure 1 shows CaPs with different morphologies synthesized within the H_2 LC phase, which are ACP spherules with a narrow size distribution (diameter 8–12nm) and ACP rods (diameter 6nm), with a specific surface area (SSA) in the range of 150–170 m^2/g. Where the ACPs synthesized using the L_α LC phase present sheet-like morphology. After the H_2O-aging route, ACP spherules evolved into elongated apatites (1.5–4 nm wide) with an ultrahigh SSA of 356 m^2/g and bone-resembling features (namely calcium and hydroxyl deficiency, carbonate substitution and poor crystallinity).

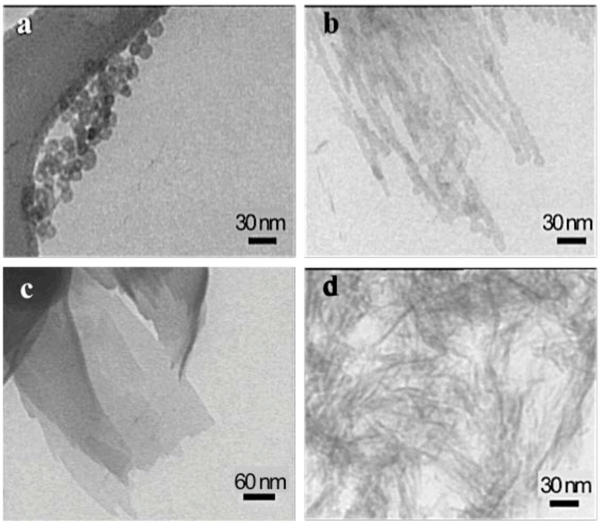

Fig. 1: TEM micrographs of (a) ACP spherules and (b) ACP rods templated by H_2 LC phase, (c) ACP sheets templated by L_α LC phase and (d) elongated apatites evolved from ACP spherules during the aging process in H_2O.

DISCUSSION & CONCLUSIONS: Within the confined space offered by the LC phases, CaPs with different morphologies (sphere, rod, sheet, wire, and even mesoporous structures) were formed. Furthermore, the conversion of ACP to crystalline apatite in aqueous media was observed as well and nanocrystalline apatite with ultrahigh SSA and bone-resembling features were prepared.

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Development of biodegradable nanocomposite scaffolds for autologous cell therapy

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INTRODUCTION: Cell scaffolds are key components in the tissue engineering paradigm, in which they can function as templates to allow new tissue growth and provide temporary structural support, while it also serves as a drug delivery system for bioactive molecules. The development of novel scaffolds with drug delivery properties is very challenging and critical to achieve the appropriate function for tissue regeneration [1]. In this study, biodegradable and biocompatible nanocomposite microparticles are developed as cell scaffolds with controlled release function in order to induce efficient autologous cell therapy.

METHODS: Nanocomposite microparticles were produced by incorporating nanoparticles into microparticles to form the nanoparticulate scaffolds. PLA nanoparticles were fabricated by the method of water/oil/water double emulsion and freeze dried before characterization and further usage. Well characterized PLA nanoparticles were suspended in mPEG-PLGA solution and subsequently loaded into the ultrasonic atomization to form the nanocomposite microparticles. The nanocomposite microparticles were analyzed with regard to the size, morphology, drug delivery property and the cell biocompatibility in the context of cell scaffolds.

RESULTS: Novel nanocomposites using biodegradable and biocompatible PLA/PLGA based materials were successfully prepared with the size around 70-80µm, which demonstrated a suitable size for the injectable cell scaffolds [2]. PLA/mPEG-PLGA nanocomposites showed controlled release property compared to the conventional microparticulate scaffolds. The morphology of nanocomposite scaffolds provided a good cell adhesion and cell biocompatibility.

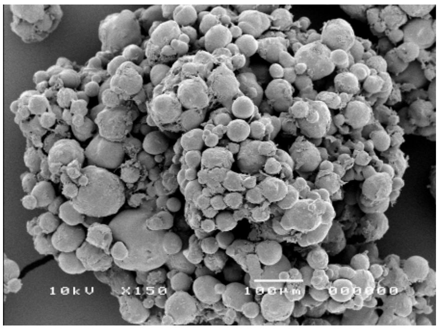

Fig. 1: cell adhesion on PLA/mPEG-PLGA nanopcomposite scaffolds.

DISCUSSION & CONCLUSIONS: Biodegradable nanocomposite scaffolds with homogenous nanoparticles incorporated have been developed in the present study. The nanocomposite scaffolds are capable of the controlled release and good cell adhesion. Therefore, these studies have demonstrated a successful design and production of nanocomposite scaffolds with functional drug delivery property that potentially can be used in autologous cell therapy.

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Biofilm susceptibility to photocatalytic dental materials

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INTRODUCTION: Biofilms are a prevalent mode of microbial life found in nature. Bacteria in biofilms are 10 to 1000 times more resistant to antibiotics than when in planktonic form, and in many cases are developing resistances to existing antibiotics. Consequently, there is a growing requirement for new strategies in biofilm elimination that do not rely on antibiotics.

Dental plaque is an example of a biofilm that can result in dental diseases such as caries. Furthermore, dental plaque is frequently associated with restorative dentistry materials, which often harbour and enhance the accumulation of bacteria.

In our former work, TiO_2 nanoparticle (NP) containing dental adhesives (referred to as NP adhesives) were shown to be bioactive in terms of mineralization. On-demand bactericidal properties could be achieved with UV-A dose of 4.5 J/cm^2. [1]

The aim of the present work was to perform an in vitro evaluation of NP adhesives for biofilm elimination through ultraviolet (UV-A) irradiation of this photocatalytic surface.

METHODS: The NP adhesives were prepared by adding 20 wt% TiO_2 nanoparticles to a light cured resin matrix of HEMA and bis-GMA polymers. Biofilm elimination testing was accomplished by irradiating the biofilm-coated surface of the NP adhesives with UV-A light, thereby generating free radicals that damage or inactivate nearby bacteria. Metabolic Activity Assays (MAA) [2] were performed to quantitatively evaluate the degree of biofilm elimination.

RESULTS & CONCLUSIONS: Results showed that a UV-A dose of approximately 6 J/cm^2 led to a 1 log reduction in the concentration of viable bacteria in the biofilm that was grown on the surface of the adhesives. As much as 7 log reduction in bacteria was achieved with a total UV-A dose of 45 J/cm2. These results show for the first time that a typical dental adhesive material containing photocatalytic TiO_2 can significantly reduce the biofilm burden when irradiated with UV-A light.

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