Novel Biomaterials and Technology for use in Regenerative Medicine/Tissue Engineering

Wednesday, 04 June 2014 09:00 - 17:00
Cineworld: The O2, London, SE10 0DX, UK

This meeting brings together the principles of engineering and life sciences in tissue development and regenerative medicine to discuss scientific research and developments of clinical applications from leading experts in the field. The newest research and developing technology will be discussed in an informal setting with plenty of networking opportunities.

This event has CPD accreditation.

This event is part of the Tissue Engineering Congress 2014 - http://tissueengineeringcongress2014.com

Meeting Chair: Dr Xuebin Yang, Head of Tissue Engineering Research, University of Leeds, UK

Talk times include 5 – 10 minutes for questions

9:30 – 10:00  Registration

10:00 – 10:10  Introduction by the Chair: Dr Xuebin Yang, Head of Tissue Engineering Research, University of Leeds, UK

10:10 – 10:35  Functional biomaterials for skeletal tissue engineering
Dr Xuebin Yang, Head of Tissue Engineering Research, University of Leeds, UK
Biomaterial is one of the key elements required for tissue engineering. There has been a push in the field of tissue engineering to develop unique biomaterials, or tissue specific scaffolds. Since tissue engineering is a dynamic process, the ideal scaffold should be biocompatible, biodegradable, bioactive and capable of supporting cell attachment, adhesion, proliferation, as well as controlling differentiation, production of extracellular matrix. This will allow tissue to regenerate, remodel and function in a physiological manner. This talk will demonstrate the use of different 3D scaffolds for skeletal tissue engineering both in vitro and in vivo, and discuss the functional elements for the design and fabrication of novel biomaterial scaffolds.

10:35 – 10:45  ORAL PRESENTATION: NATURALLY DERIVED HYDROGEL MATERIALS FOR SCAFFOLDING VIA TWO-PHOTON POLYMERIZATION (2PP)
Olga Kufelt, Camilla Sehring, Ayman El-Tamer, Marita Meissner, Sabrina Schlie-Wolter, and Boris N. Chichkov
Laser Zentrum Hannover e.V., Nanotechnology Department, Hollenthallee 8, 30419 Hannover, Germany

10:45 – 10:55  ORAL PRESENTATION: THE CONNEXIN 43 EXPRESSION IN VASCULAR SMOOTH MUSCLE IS POTENTIATED BY DYNAMIC STIMULATION; POSSIBLE APPROACH HOW TO STIMULATE THE INTERCELLULAR COMMUNICATION
Jana Musílková 1, Tomáš Riedel 2, Eduard Brynda 2, Lucie Bačáková 1
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Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic,
Heyrovsky Sq. 2, 162 06 Prague-6, Czech Republic

10:55 – 11:20 Biological scaffolds for tissue engineered organ replacement
Dr Steve Bloor, Videregen, UK
There is a current and emergent need to expand organ transplant availability. Tissue engineered regenerative therapies using decellularised biological scaffolds seeded with autologous cells have the capability to restore organ function, increase quality and length of life and reduce healthcare costs. Videregen’s core technology uses decellularised organ scaffolds seeded with autologous cells to create non-immunogenic and personalised organ replacements. Tissue engineered tracheal replacements have already demonstrated proof of clinical principle and initial safety in compassionate use clinical cases. This lead indication is scheduled to progress into formal clinical trials within the next 12 months. There is additional pre-clinical proof of concept in a second indication which shows that decellularised rat bowel can be recellularised with stem cells leading to regeneration of differentiated organ tissue. Translation of the rat studies into a porcine model show that implantation of a clinically relevant small bowel segment leads to successful reperfusion of the vascular system of the bowel, a critical success criteria and milestone.

11:20 – 11:45 Speakers’ photo then mid-morning break and poster exhibition and trade show
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11:45 – 12:10 Assessing the Mechanical Properties of Biomaterials for Tissue Engineering Under Physiological Loading Conditions
Dr Stefanie Biechler, Bose ElectoForce, Germany
The degeneration of tissue, resulting in loss of biological function, has driven efforts in the field of tissue engineering to grow replacement tissues through mechanical and biochemical stimulation. In the case of cell-seeded scaffolds, it is imperative that a support material be selected to have appropriate mechanical properties. However, many of these materials are characterized under non-physiological loading conditions. In this study, various biomaterials, including hydrogels for cartilage tissue engineering and electrospun tubes for vascular applications, were characterized under physiologically relevant conditions selected to mimic the dynamic nature of the body.

Dr Florelle Gindraux, Université de Franche Comté, France

12:35 – 12:45 ORAL PRESENTATION: BIOINDENTER: A NEW INSTRUMENT FOR INDENTATION OF SOFT TISSUES AND BIOMATERIALS  G. Weder, M. Liley and H. Heinzelmann, CSEM, Swiss Center for Electronics and Microtechnology, Jaquet-Droz 1, 2002 Neuchâtel, Switzerland

12:45 – 12:55 ORAL PRESENTATION: FIBRIN AND COLLAGEN STRUCTURES ON NANOFIBROUS MEMBRANES AS CARRIERS FOR SKIN CELLS
M. Bacakova 1, T. Riedel 2, D. Stranska 3, E. Brynda 2, L. Bacakova 1
12:55 – 14:05 **Lunch, poster exhibition and trade show**
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14:15 – 14:25 **ORAL PRESENTATION: ENHANCED BIOACTIVITY OF TITANIUM BY LASER-GENERATED ‘LOTUS’-TOPOGRAPHY FOR ORTHOPAEDIC APPLICATIONS** Sabrina Schlie-Wolter, Elena Fadeeva, Andrea Deiwick, Boris Chichkov
* Laser Zentrum Hannover e.V.; Department of Nanotechnology; Hollerithallee 8; 30419 Hannover; Germany*

14:25 – 14:50 **Direct cytotoxicity evaluation of biomaterials by micro/nano calorimetry: a new method for materials biocompatibility evaluation**
*Dr Ali Doostmohammadi*, Head of Materials Department, Shahrekord University, Iran
The evaluation of biocompatibility is one of the most important assessments to be performed prior to clinical use of biomaterials. There is a need for a convenient method for screening of biocompatibility and cytotoxicity (as a criterion of biocompatibility), a method that can directly evaluate cell growth as well as cell adhesion to biomaterials surfaces. Isothermal micro-nano calorimetry (IMNC) is capable of measuring the heat production or consumption rate in the microW range with a calorimeter operating at nearly a constant temperature. Therefore, this technique allows direct and continuous monitoring of the metabolic activity of living cells.

14:50 – 15:10 **Afternoon Tea, last poster session and trade show**
Professor Gou-Jen Wang, National Chung-Hsing University, Taiwan  
A specifically designed scaffold can enhance the division, proliferation, and differentiation of embryonic stem cell. In this study, various porous PLGA/Gelatin scaffolds coated with mixed solution of gelatin and vascular endothelial cell conditioned medium (ECCM) on the scaffold and pore inner surfaces are proposed. The PLGA/gelatin composited scaffold serves as a control release platform to induce the differentiation of mouse embryonic stem cell into endothelial-like cell. Without any additional growth factor, the starting differentiation time point of mouse embryonic stem cell cultured on the proposed control release scaffolds can be reduced to 1/2 of that of the conventional approaches.

15:55 – 16:25  Design of Polymeric Biomaterials for Musculoskeletal Tissue Repair  
Associate Professor Fariba Dehghan, Director of bioengineering and Postgraduate coursework, The University of Sydney, Australia  
Hybrid biomaterials fabricated from ceramics, polymers and biopolymer are deemed to be the material of choice due to their tunable physicochemical properties. In our studies, we prepared hybrids from natural and synthetic polymers with bioactive glass by formation of covalent bond. It was demonstrated that the presence of chemical bonds between the polymers and bioactive glass eradicates the issue of phase-separation and enhances the uniform distribution of components in the structure of these materials. Assessment of in vitro bioactivity of these samples showed that a homogenous apatite layer was formed on the surface of hybrid scaffolds. Furthermore, it was shown that the presence of chemical bond resulted in uniform degradation of different components in hybrids. The fabricated materials could be considered as viable candidates for bone regeneration. We also synthesized thermoresponsive copolymers that chemically bond with primary amine groups of proteins. The copolymers-protein solution was injectable through 21G needle and converted to hydrogel within 2-10 minutes by increasing the temperature to 37°C. The gelation time of these hydrogels was favorable for clinical applications. They retained in vivo for more than a month due to the presence of covalent bonds between the polymer and protein. The fabricated hydrogels, therefore, are deemed to have high potential for various biomedical applications such as in vivo cartilage and bone regeneration.

16:25 – 16:35 ORAL PRESENTATION: THE NOVEL BIOACTIVE GLASS COATINGS FOR BONE TISSUE ENGINEERING: AN IN VITRO STUDY  

1,2,3 Lotfibakhshaiesh, N*; 4Hill, R; 2,3Stevens, M M  
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16:35 – 17:00  Design of Novel 2D/3D Biocompatible Polymers Based on Self-organized Water Structure for Medical Devices and Tissue Engineering
The design of biocompatible 2D surfaces and 3D nano/micro topographies based on self-organization inspired by biology has a variety of potential applications in medical devices and tissue engineering. Many polymers have been studied to obtain biocompatible surfaces, and several mechanisms have been proposed for the biocompatibility of polymers \[1\]. It has been pointed out that biocompatibility depends on the various physicochemical properties of the material surface. We have reported that 1) bio compatible 2D surface using poly(2-methoxyethyl acrylate) (PMEA) \[2-11\] and 2) honeycomb-patterned 3D films with regular interconnected pores that is formed by self-organization \[13-15\].

1) We found that hydrated PMEA has unique water observed as a cold crystallization (CC) of water in differential scanning calorimetry (DSC), ATR-IR, NMR, and AFM. This CC is interpreted as the ice formation at low temperature, that belongs to the intermediate water in PMEA. The CC peak was observed for hydrated PEG, poly(2-methacryloyloxyethyl phosphorylcholine)(PMPC), PVP, as well as various proteins, polysaccharides and DNA (RNA), which are well-known as biocompatible polymers. The amount of the intermediate water of PMEA-analogous polymers affected the stem cell and cancer cell adhesion. The control of stem cell cancer cell adhesion by biocompatible polymers will open the way for a new cell therapy called as personalized medicine. 2) We also found that the topography of the 3D films in contact with cancer cells has a potential anticancer effect. In these results, the 3D films could regulate cell adhesion, morphologies and functions in the absence of growth factors and anticancer drugs. A bile-duct stent which is covered by the 3D films is now commercially available in the world clinical market. We will highlight that 1) the reasons for the selective adhesion of stem and cancer cells on PMEA by comparing the structure of water in hydrated PMEA to the water structure of other polymers and 2) the reasons that 3D films exerted a strong influence on normal, cancer and stem cells morphology, proliferation, differentiation, cytoskeleton, focal adhesion, and functions including matrix production profiles.

17:00  Chairman’s Summing Up and Close of Meeting

Registration Website: [http://www.regonline.co.uk/TissueEng2014](http://www.regonline.co.uk/TissueEng2014)

About the Chair

**Xuebin Yang**, the head of tissue engineering research at Leeds Dental Institute, UK, received his first degree in medicine, and has 12 years’ clinical expertise in orthopaedics and medical devices design (3 patents). He completed his PhD degree on stem cell therapy and bone tissue engineering at the University of Southampton (2002). In 2004, he moved to Leeds and established a tissue engineering initiative. To date, he has supervised 14 MSc students, 20 PhD students and 8 fellows in the use of different biomaterials for skeletal tissue engineering (include bone, cartilage, dental pulp and complex tissues) in both medical and dental applications. In particular, Dr Yang has contributed to the design of many different biomaterial scaffolds for tissue engineering applications.

About the Speakers

**François A. Auger** obtained his MD in 1978, followed by specialization training and a fellowship at the NIH in Washington, DC. Since 1985, he has been the director and founder of the Experimental Tissue Engineering Laboratory (LOEX) which is located in the CHU de Québec (CHA) affiliated with Laval University, where he presently is full professor of surgery. The LOEX was the first research group in Canada dedicated to the reconstruction of tissues by human cell culture. Dr Auger has overseen and
participated in the diversification of his team’s research interests into various domains including skin and vascular tissue engineered substitutes.

**Stefanie Biechler** received her PhD in Biomedical Engineering from the University of South Carolina for her work investigating the influence of flow-induced forces on the development of heart valves in an in vitro system of cardiac development. Her experience in computational modeling, materials science, and cellular biology has translated into a role as an Applications Engineer to support research in tissue engineering and regenerative medicine.

**Ali Doostmohammadi**, the head of materials department at Shahrekord University, IRAN, has completed his Ph.D in Biomaterials at Isfahan University of Technology (IUT) (2010). In 2011, He moved to Shahrekord university (SKU). The central area of his research is nanobiomaterials, bioceramics, bioactive glasses and their applications in tissue engineering. To date, Dr Doostmohammadi has published more than 30 papers in reputed journals and supervised about 20 research projects in biomaterials and tissue engineering.

Gou-Jen Wang received the B.S. degree on 1981 from National Taiwan University and the M.S. and Ph.D. degrees on 1986 and 1991 from the University of California, Los Angeles, all in Mechanical Engineering. Following graduation, he joined the Dowty Aerospace Los Angeles as a system engineer from 1991 to 1992. Dr. Wang joined the Mechanical Engineering Department at the National Chung-Hsing University, Taiwan on 1992 as an Associate Professor and has become a Professor on 1999. From 2003-2006, he served as the Division Director of Curriculum of the Center of Nanoscience and Nanotechnology. From 2007 to 2011, he has been the Chairman of the Graduate Institute of Biomedical Engineering, National Chung-Hsing University, Taiwan. On 2008, he served as the Conference Chair of the Microfabrication, Integration and Packaging Conference (April/2008, Nice, France). From 2009, he is a Committee member of the Micro- and Nanosystem Division of the American Society of Mechanical Engineers. His research interests include MEMS/NEMS, biomedical micro/nano devices, nano fabrication, and tissue engineering.

**Steve Bloor** has over 20 years’ experience in medical devices and regenerative medicine, with key expertise and leadership in strategic R&D, clinical compliance and regulation specific to these areas having worked in US multinationals (J&J, Covidien) and UK start-up companies. Steve is currently CEO at Videregen Ltd, a UK regenerative medicine company focussed on tissue engineered organ replacements. Formerly CSO at Tissue Science Laboratories (TSL) plc. and VP R&D at Covidien.

**ORAL PRESENTATIONS**

**BIOINDENTER: A NEW INSTRUMENT FOR INDENTATION OF SOFT TISSUES AND BIOMATERIALS**

G. Weder, M. Liley and H. Heinzelmann

*CSEM, Swiss Center for Electronics and Microtechnology, Jaquet-Droz 1, 2002 Neuchâtel, Switzerland*

Tissue biomechanics is an emerging field with the potential to make a significant contribution to the study of human diseases. Viscoelastic characterization of soft biological materials on the micrometer scale is bringing new insights into pathological states such as atherosclerosis, osteoarthritis, cancer progression as well as into tissue engineering. Until recently this field has been largely neglected for two main reasons. Firstly, soft biological tissues exhibit complex anisotropic and nonlinear viscoelastic mechanical properties. Secondly, biological tissues need to be analysed under conditions that are as close as possible to the *in vivo* situation.
The viscoelastic properties of biological tissues are measured by indenting the sample and by analysing indentation-force versus indentation-depth of the load and unload cycle. Two types of tool are currently available. On the one hand, atomic force microscopes (AFM) are used to probe single cells with small applied forces (in the pN to nN range). On the other hand, nanoindenters – originally developed to indent thin and hard surfaces - can be used to measure materials properties with applied forces in the mN range and higher. Neither AFM nor nanoindenter instruments are ideal for soft biological tissues, which require forces in the μN range and a physiological environment. To fill this gap, a new instrument has been developed for measurement of the mechanical properties of soft biological tissues and biomaterials: the Bioindenter. It can measure the elastic moduli from 1 kPa to 400 MPa. The Bioindenter is equipped with a Biochamber that includes a temperature-controlled fluidic chamber based on disposable Petri dishes. In addition to epi-illumination, a compact inverted phase contrast microscope has been developed and implemented for the observation of semi-transparent samples with poor contrast such as multi-layered cells or microtissues. The microscope is fluorescence-compatible and allows an observation of the sample during the indentation process. The new instrument has been validated for early cartilage and tendon repair. Elasticity of goat articular cartilage was measured and compared between healthy regions and early repair tissue after 3 months. Young’s modulus mean values were calculated using the Hertz model. The Bioindenter was also used to develop biological scaffolds for tendon. 3D scaffolds were prepared by the removal of cellular components to minimize the risk of host rejection while maintaining as much as possible the mechanical properties. A new instrument was developed for indentation of biological tissues and biomaterials, bridging the gap between AFM and nanoindenters. Studies of healthy and diseased ex vivo tissues as well as engineered tendon replacements demonstrated the capability of the Bioindenter to measure the local mechanical properties of soft materials under physiological conditions. The Bioindenter combination of imaging and mechanical analysis is one step closer to understanding the incredibly complex structure and mechanical properties of soft biological tissues. Corresponding author: gilles.weder@csem.ch

SURFACE-MODIFIED Ti-6Al-4V ALLOY FOR OSTEOGENIC IN VITRO STUDY
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We have studied the interaction of human bone cells in vitro with samples of Ti–6Al–4V, i.e. an alloy commonly used for construction of bone implants, modified by electric discharge machining (EDM), chemical milling (CM) or shot-peening (SP), and by combinations of these treatments. The reason for these modifications was to improve mechanical and other physical and chemical properties of the material, but an increase in the attractiveness of the material for osteoblast adhesion, growth and osteogenic cell differentiation was also expected. Four groups of Ti–6Al–4V samples with the following modifications were prepared:

#1. Polished Ti–6Al–4V samples were used as a standard (ST)
#2. EDM
#3. EDM + CM (in Kroll’s reagent)
#4. EDM + SP with ceramic balls (ZrO₂ and SiO₂ mixture) 125-250 μm in diameter
#5. EDM + CM + SP

The materials were sterilized in hot-air sterilizer, inserted into 12-well cell culture polystyrene plates and seeded with Saos-2 cells (43,000 cells in 3 ml of the McCoy’s medium with 10% of fetal bovine serum).
We found that chemical milling (rather than shot peening) played a key role in improving the adhesion and growth of bone cells. The cells on these materials had numerous and well-developed talin-containing focal adhesion plaques, which is a sign of their strong adhesion to the material. These cells also had an increased activity of mitochondrial dehydrogenases, as measured by the XTT test, and a relatively high DNA content, which indicated a high population density of these cells. These favourable results were probably due to the elimination of potentially cytotoxic remnant particles persisting on the surface after EDM treatment, reduction and rounding of surface irregularities, and particularly the oxidation of the material surface, which increased the surface polarity and wettability. Nevertheless, subsequent shot-peening significantly improves the fatigue endurance of the material. As revealed by the measurement of DNA content, which is an indirect indicator of cell number, the EDM + CM + SP samples contained the highest number of cells on day 7 after seeding.

An interesting result was obtained when the expression of markers of osteogenic differentiation was studied in Saos-2 cells by real-time PCR. On day 14 after seeding, the highest expression of collagen I, i.e. and early marker of osteogenic cell differentiation, was found in cells cultured on EDM and EDM+SP samples. This result can be explained by the highest roughness of these samples, measured by the Ra parameter. It has been repeatedly reported that high material surface roughness is associated with lower cell proliferation, but increased osteogenic cell differentiation. The expression of alkaline phosphatase and osteocalcin, i.e. an intermediate and late marker of osteogenic cell differentiation, respectively, were similar on all tested samples.

Thus, it can be concluded that the Ti–6Al–4V treated with chemical milling (EDM + CM, EDM + CM + SP) are suitable in applications where the growth of osteoblasts is needed, while samples treated with EDM or EDM + SP are good for induction of the osteogenic cell differentiation.

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ENHANCED BIOACTIVITY OF TITANIUM BY LASER-GENERATED ‘LOTUS’-TOPOGRAPHY FOR ORTHOPAEDIC APPLICATIONS
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Advanced functional biomaterials have to combine following aspects: appropriate mechanical properties, low immune rejection, reduced risk of infections and a high biocompatibility. For orthopaedic application this biocompatibility depends on strong anchorage of bone cells to reduce stress shielding effects and enhanced osseointegration classified via osteogenic differentiation to stimulate bone regeneration. Titanium, the material of choice for orthopaedic applications due to its outstanding mechanical properties similar to native bone, does not provide appropriate biological performances. Therefore, an optimization of the titanium surface is required.

Surface structuring is one promising approach to improve material's bioactivity. Thereby, the usage of ultrashort-pulse laser ablation is very advantageous, since complex surface features in micro- and nanoscale can be produced in almost all solid materials. Additionally, the size dimensions of each surface type can be varied by changing the laser processing parameters. Laser structuring always causes an increase of material wettability enabling the fabrication of superhydrophobic surfaces. Further coating with extracellular matrix components does not negatively affect the texturing. [1]

To mimic the hierarchical architecture of native bone, we create so-called ‘lotus’-features in titanium, which consist of microsized convex structures covered with nano-roughness. We demonstrate that these features selectively inhibit the adhesion and proliferation of fibroblasts, which participate in scar tissue formation after implantation. At the same time osteoblasts are not negatively affected [2]. Furthermore, the features control the colonization of pathogenic bacteria like S. aureus and P. aeruginosa [3]. In comparison to collagen type I coating, we demonstrate that the ‘lotus’-features in titanium significantly enhance bone binding activity. Additionally, osteogenic differentiation of human
adipose-derived stem cells is improved, especially calcium mineralization, which is considered as the strongest differentiation marker of functional bone [4].

In a sum, laser-generated ‘lotus’-topographies fulfil all four criterion of an advanced functional biomaterial in vitro and hold a great promise for future orthopaedic and dental applications.

Keywords: Functional biomaterial, osteoinduction, stem cells, tissue engineering


NATURALLY DERIVED HYDROGEL MATERIALS FOR SCAFFOLDING VIA TWO-PHOTON POLYMERIZATION (2PP)
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Two-photon polymerization (2PP) is a prospective technique which allows the fabrication of complex 3D microstructures by direct laser writing in the volume of a photosensitive material. The fabrication process is based on multiphoton absorption of femtosecond laser pulses inducing a highly localized photochemical reaction, which leads to polymerization of the material. In the last years, 2PP has emerged as a promising technique in tissue engineering and basic research in biomaterials science [1]. So far, the selection of suitable prepolymers is limited to commercially available synthetic materials including various acrylate-based precursors. We have prepared a series of photosensitive naturally derived hydrogel materials for tissue engineering applications. Hydrogels are able to mimic the basic three-dimensional (3D) biological, chemical and mechanical properties of native tissues and therefore could deliver well adapted materials for cell seeding and encapsulation as well as for implantation. Due to their poor mechanical properties hydrogel structuring remains a major challenge. The chemical modification of hydrogels like hyaluronan [2], chitosan [3] and alginate allows the application of the 2PP-technique to such natural materials and thus, the fabrication of 3D hydrogel constructs with well-defined architectures and porosity to perform cell studies in a controlled 3D environment.

The modification of the hydrogels was performed using glycidyl methacrylate or methacrylic anhydride chemistry, respectively. 1H NMR studies confirmed the introduction of vinyl groups into the hydrogel backbones. In case of chitosan, we developed a water-soluble and photosensitive derivative. According 1H NMR studies and 2,4,6-trinitrobenzenesulfonic acid assay, photosensitive hydrogels with different degrees of modification were synthesized. To modulate the mechanochemical properties of the generated hydrogels, they were combined with poly(ethylene glycol) diacrylate (PEGDA) as a model component providing the option to produce any blend material in situ. According to rheology studies, elasticity and stiffness of the obtained hydrogels and hydrogel PEG blends can be adjusted for the targeted cell application. LDH and Alamar Blue cytocompatibility studies have shown the biocompatibility of the cross-linked materials and blends. To further enhance the biocompatibility, the hydrogels were functionalized with growth factors (EGF, VEGF) via coupling with N-succinimidyl acrylate. Applying 2PP, well elaborated hydrogel and PEG hybrid microstructures with predefined pore sizes were obtained. Using this technique, hydrogel microstructures with predefined geometry, pore size and porosity can be produced.
Concluding, we have prepared a series of photosensitive hydrogels which can be used to fabricate identical bioactive 3D matrices for systematic cell investigations including proliferation, migration and differentiation.


PROMOTING ENGRAFTMENT OF TRANSPLANTED NEURAL STEM CELLS/PROGENITORS USING SCAFFOLDS TETHERED WITH GDNF AND THE CHARACTERISATION OF STABILITY AND BIO-FUNCTIONALITY

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Given the brain’s limited capacity for repair, new and innovative approaches are required to promote regeneration. Whilst examples of neural transplantation have been demonstrated, major limitations in the field include poor cell survival and integration. This in part is due to the non-conducive environment of the adult brain – failing to provide adequate chemical and physical support for new neurons. Here we examine the capacity of polycaprolactone (PCL) scaffolds combined with immobilization of neurotrophin, glial derived neurotrophic factor (GDNF), to improve graft survival and integration. Immobilization of GDNF was confirmed using Enzyme-linked immunosorbent assays (ELISA) and immunohistochemistry, prior to and at 28days post-implantation, demonstrating long-term delivery of GDNF. Despite the benefits of long-term delivery system, little knowledge exists regarding the stability and functionality of tethered proteins over time. Here we demonstrated the stability, degradation, longevity and activation of intercellualr pathway and biofunctionality of tethered GDNF, which is known to influence neuronal survival, differentiation and neurite morphogenesis. ELISA revealed that tethered GDNF, remained present on the scaffold surface for 120 days, with no evidence of protein leaching or degradation. The tethered GDNF protein remained functional and capable of activating downstream signalling cascades for up to 3 days, as revealed by its capacity to phosphorylate intracellular Erk in a neural cell line (SN4741). In vitro, PCL enriched for neural stem cells in primary cortical cultures, compared to cultureware. Subsequent implantation of cells, cells+PCL or cells+PCL-GDNF indicated that PCL-GDNF was capable of supporting grafts, as demonstrated by the increased graft survival and penetration of cells and neurites into the materials. In conclusion, these findings provide evidences of the stability, longevity, activation of intracellular pathway and the biofunctionality of tethered GDNF. This study demonstrated that modified scaffolds with GDNF can support grafted neurons - promoting survival, penetration/integration and encouraging neurite growth.

THE NOVEL BIOACTIVE GLASS COATINGS FOR BONE TISSUE ENGINEERING: AN IN VITRO STUDY

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Introduction
Metallic prostheses are widely used to treat joint and skeletal injuries and disease. However, metal alloy implants can sometimes fail due to complications of fibrous encapsulation and poor stress transfer between the bone and the implant. Bioactive glass (BG) coatings may promote the formation of a strong bond with living bone tissue thus decreasing the likelihood of fibrous encapsulation and have the added benefit that their dissolution ions stimulate cell activity [1,2]. Strontium (Sr) ranelate, a drug used to treat and prevent osteoporosis, works via the action of Sr ions which stimulate the formation of new bone and prevent osteoclast-mediated resorption [3]. We have previously shown that Sr-substituted BGs promote osteoblast activity in vitro [4] and explored the effect of altering phosphate content on the material structure of soda-lime-phosphosilicate glasses [5]. The effect of increasing phosphate content in Sr-substituted BG on cultured osteoblasts, however, remains unexplored. Here, we created Sr-substituted BG coatings with a range of phosphate contents and thermal expansion coefficients that matched that of Ti alloy, producing materials that combine the bone remodelling benefits of Sr and BG with phosphate to mediate pH changes which can affect cell viability. In the study presented here we report the characterisation of these multicomponent BG coatings in terms of their bioactivity and interaction with cells.

Materials and Methods
Bioactive glasses in the system SiO$_2$-MgO-Na$_2$O-K$_2$O-ZnO-P$_2$O$_5$-CaO in which 10% of the Ca was replaced by Sr and the P$_2$O$_5$ content was increased from 1.07 to 6.42 mol% were produced by a melt quench route. Sufficient cations were added to ensure charge neutrality in the PO$_4^{3-}$ complex formed. Simulated body fluid (SBF) was prepared according to Kokubo [6]. Glass particles (<38 micrometer) were immersed for up to 28 days and agitated at 60 rpm at 37°C. At indicated time points samples were filtered and dried for X-Ray Diffraction (XRD) analysis. Culture media containing ions from glasses were created by incubating 1.5g/L of glass powder (<38 micrometer) in RPMI 1640 on a roller for 4 hours at 37°C and then passed through a 0.2 micrometer filter. This media was then supplemented with 10% (v/v) foetal bovine serum (FBS), 2mM L-glutamine and 1% (v/v) penicillin/streptomycin. The human osteosarcoma cell line, Saos-2, was seeded at 30,000 cells/cm$^2$ in conditioned medium and cultured for up to 28 days. On days 1, 14, 21 and 28 cell metabolic activity was measured using the tetrazole MTT as an indicator of cell proliferation. Glasses were coated on the surface of Ti6AL4V coupons with an enameling technique. 10,000 Saos-2/cm$^2$ were seeded on BG coatings and viability was assessed after 1, 7 and 14 days with a LIVE/DEAD stain. Some glass coatings cultures were also fixed, gold coated and viewed on a Leo 1525 Gemini SEM.

Results and Discussion
BG with high P$_2$O$_5$ content forms more apatite after immersion in SBF for 4 weeks than BG with low P$_2$O$_5$ content, as examined by XRD. MTT activity in Saos-2 cells treated with dissolution ions from BG increased in all samples with time in culture. MTT activity was also significantly greater ($p<0.01$) in cells treated with dissolution ions from 4.28 and 6.24 mol% P$_2$O$_5$ BGs as compared to controls at day 28. LIVE/DEAD staining indicated that all coating materials were not cytotoxic. SEM imaging demonstrated that the BG coating encouraged cell attachment and that cells spread well over the surface.

Conclusion
With increasing P$_2$O$_5$ content in the series of Sr-substituted BG, Bragg peaks in XRD traces associated with apatite crystallisation increase suggesting the glass becomes more bioactive. Apatite formation on the coating surface is an essential factor for bone bonding as the more apatite that forms on the glass
coating the more bone bonding will be expected. Adding $P_2O_5$ to the glass composition in a controlled range prevents extreme pH rises, which can affect cell viability and proliferation.

References

FIBRIN AND COLLAGEN STRUCTURES ON NANOFIBROUS MEMBRANES AS CARRIERS FOR SKIN CELLS
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Nanofibrous materials are increasingly applied in the recent tissue engineering. They better simulate the structure of fibrous components of natural extracellular matrix than conventional flat or microstructured materials and thus they are considered to be more attractive for cell adhesion and growth. Nanofibrous membranes coated with biomolecules which are normally present in the natural skin (collagen, hyaluronan) or occur during wound healing (fibrin) could be used in skin tissue engineering for constructing a bilayer of fibroblasts and keratinocytes. These membranes will separate both cell types but due to the pores, these structures will ensure physical and humoral communication of the cells, thus the layer of fibroblasts can serve as nutrient feeder for keratinocytes like in the natural skin.

In this study we used nanofibrous membranes made of biodegradable polylactide (PLA) that can be slowly resorbed in the organism and finally fully replaced by regenerative tissue. The PLA membranes were prepared using the novel Nanospider needleless electrospinning technology. We coated the membranes with fibrin, fibrin with fibronectin (cell adhesion-mediating extracellular matrix protein), collagen I or collagen I with fibronectin, and then we evaluated the material interactions with human dermal fibroblasts and human dermal keratinocytes of the line HaCaT.

Results indicated that PLA nanofibrous membranes promoted adhesion and growth of the skin cells. Fibrin and collagen structures on PLA membranes further improved adhesion, proliferation and metabolic mitochondrial activity (determined by MTS assay) of the skin cells. The human dermal fibroblasts preferentially adhered and were more spread on the membranes coated with fibrin, fibrin with attached fibronectin on its surface or collagen I with fibronectin than on the membranes coated only with collagen or on the membranes in pristine form. Moreover, the metabolic activity of human dermal fibroblasts was the highest on the membranes coated with fibrin or fibrin with fibronectin. The membranes coated with collagen I or collagen I with fibronectin promoted spreading of the HaCaT keratinocytes and increased the cell metabolic activity in comparison with pristine membranes or membranes coated with fibrin or fibrin with fibronectin. Viability (determined by a Live/Dead assay) of the fibroblasts and the keratinocytes on the membranes was almost 100 % on all samples.

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THE CONNEXIN 43 EXPRESSION IN VASCULAR SMOOTH MUSCLE IS POTENTIATED BY DYNAMIC STIMULATION; POSSIBLE APPROACH HOW TO STIMULATE THE INTERCELLULAR COMMUNICATION

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Connexin 43 is the main cardiac connexin found in ventricular myocardium. Connexins, i.e. gap junction proteins, are a family of structurally related transmembrane proteins that assemble the vertebrate gap junctions. They serve as intercellular channels which enable the neighbouring cells in a tissue to share ions, second messengers, and small metabolites. Thus, these structures provide the intercellular communication.

Vascular tissue cells respond to changes in the mechanical load imposed on them. That is why we evaluated the influence of uniaxial stretching on the mRNA expression of connexin 43 in vascular smooth muscle cells (VSMC) under various conditions. The mRNA expression of connexin 34 was tested by qRT PCR using hydrolysis TaqMan probes. The experiments were performed in duplex with β-actin as a reference gene. The cells were also stained by immunofluorescence methods to show the changes in the α-actin cytoskeleton, i.e. a marker of VSMC differentiation towards the contractile phenotype, and in the molecular pattern of connexin after stretching.

Experiments were performed using the Strex equipment (B Bridge International, Ltd). Flexible silicone chambers were coated with type I collagen and fibronectin. VSMC were seeded in a density of 45 000 cells/cm² for qRT PCR experiments. After a 2-day static culture, the VSMC were subjected to stretching at frequency of 0.5Hz and amplitude of 5% for 30, 60, 90, 120 min and 2 days. Moreover after 2 days, the periodical stretching frequency was changed to 1Hz and measured again in intervals 15, 30, 60, 90 min and 24 hours. At the same time, the cells were cultivated under static conditions. An additional experiment in that we changed conditions to amplitude of 10% was performed by the same way.

We found that the periodical uniaxial stretching caused the rise of Connexin 43 expression. At a lower degree of stimulation (frequency of 0.5 Hz and amplitude of 5%), the maximal expression was about 3 fold in comparison with the static control, with the maximum after 60 min of stimulation. For longer time periods of stretching, the expression of Connexin 43 decreased later again. After additional stretching at physiological conditions (frequency of 1 Hz and amplitude of 5%), the mRNA expression rose immediately almost 5 fold, with the maximum in the first 30 min. Uniaxial stretching at amplitude of 10% does not affect the Connexin 43 expression at any frequency.

It can be concluded that dynamic stimulation supports the intercellular communication of VSMC, and by this way forms their phenotype more convenient for the tunica media reconstruction on vascular replacements. The rise of Connexin expression depends on the dynamic culture conditions. Our results indicate that the maximal stimulation appears in conditions of consecutive dynamic load with appropriate amplitude of 5%.

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POSTER PRESENTATIONS

LIGHT OPTICAL AND ELECTRON MICROSCOPIC CHARACTERISTICS OF TISSUE EQUIVALENTS BASED ON MULTIPOTENT MESENCHYMAL STROMAL CELLS AND COLLAGEN MATRIX
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Adipose tissue is an available source of both self-specific and allogenic multipotent mesenchymal stromal cells (MMSC). Therefore, the use of adipose derived MMSC with the view to fabricate tissue equivalents for regenerative medicine is a topical and promising field of biotechnolog. The purpose of our work was to carry out light optical and electron microscopic studies of MMSC isolated from adipose tissue and grown on the matrix manufactured from bovine collagen. The work was performed within the scope of the subproject “Biological coverings on the basis of natural polymers for treatment of burn and surgical wounds” funded as part of the Technology Commercialization Project supported by the World Bank and the Government of the Republic of Kazakhstan. MMSC from adipose tissue were grown on collagen matrix using Mesencult medium (Stemcelltechnology, Canada). A morphological examination of sections on the 9th day of culture revealed the presence of fibroblast-like cells on the collagen matrix. Basophilic adipose tissue (AT) MMSC attached themselves to collagen fibers. Some cells contained lipid inclusions. Under an electron microscope AT MMSC had narrow spindle-like shape with simplified structure characteristic of poorly differentiated mesenchymal cells. The nuclei contained electron-dense chromatin, however, nuclei with electron transparent karyoplasm and dense nucleoli were also observed. The cytoplasm was abundant with free ribosomes and polyribosomes, filaments and microtubules. In the central part of the cell there were narrow or enlarged cysternae of rough endoplasmic reticulum with fine-grained material in the lumen, smooth membranes of Golgi complex and multiple vesicles. Mitochondria were sparse and large with fine-grained matrix of high electron density and transverse closely placed cristae. Spirally twisted myelin bodies were observed. The cells were tightly attached to the matrix fibrils with typical cross striation.

Conclusion
1. Bovine collagen-based matrix may be used for culturing of adipose derived MMSC.
2. AT MMSC have fibroblast-like structure and attach themselves to collagen matrix fibers.
3. Electron microscopic study has shown that AT MMSC grown on collagen matrix have well-developed intracellular structure.
4. The matrix on the basis of bovine tendon collagen can be employed for fabrication of AT MMSC-based tissue equivalents.

BIOMATERIALS OF H- AND O-TERMINATED NANOCRYSTALLINE DIAMOND FILMS
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Nanocrystalline diamond (NCD) films are promising materials for tissue engineering, especially for bone implants coating, due to their biocompatibility, chemical resistance and mechanical hardness. Nanostructure and morphology of the NCD films can efficiently mimic the properties of natural tissues, and thus they support the cell adhesion, proliferation and differentiation. In addition, the NCD wettability
can be tailored by grafting specific atoms and functional chemical groups (e.g., oxygen, hydrogen, amine groups, etc.) which influence the adsorption and final geometry of proteins, and thus the behaviour of cultivated cells. Therefore, the NCD films are proposed as multifunctional materials for fundamental studies on the growth and adhesion of osteoblasts on bone implants, which is particularly our interest.

The NCD films used in this study were grown on silicon substrates by microwave plasma-enhanced chemical vapor deposition. The quality of the grown NCD films was investigated by Raman spectroscopy, scanning electron microscopy and atomic force microscopy. In order to control the hydrophobic or hydrophilic character, the NCD film surfaces were grafted by hydrogen (H-termination) or oxygen (O-termination) atoms. The influence of surface termination on the surface wettability (wetting contact angle) was characterized by reflection goniometry using droplet of deionized water. The primary human osteoblasts, mesenchymal stem cells (MSCs) and osteoblast-like Saos-2 cells were used for biological studies on H- and O-terminated NCD films. The cell adhesion and spreading was analysed by the visualisation of focal adhesion proteins (talin, paxillin) and actin fibers. Expression of markers of osteogenic cell differentiation (alkaline phosphatase, osteocalcin, collagen I) was monitored by the reverse transcription and Real-time PCR method, and also by immunostaining of expressed proteins and image analysis. The extracellular matrix production and composition, i.e. collagen content, calcium content and activity of alkaline phosphatase, were also quantified. Native type I collagen fibres were visualised by two-photon excitation microscopy and second harmonic generation imaging, together with immunostaining and fluorescence microscopy.

We found that primary human osteoblasts cultivated on the O-terminated NCD films exhibited better adhesion compared to the H-terminated NCD films. Also the expression of osteogenic cell markers such as collagen and osteocalcin was higher in MSCs on the O-terminated films. The mature collagen fibers were detected in Saos-2 cell layers on both H- and O-terminated NCD films; however, the quantity of collagen in extracellular matrix was higher on O-terminated NCD films. The amount of deposited calcium and alkaline phosphatase activity were also significantly higher in Saos-2 cell layers on O-terminated NCD films. In conclusion, the higher wettability of the O-terminated NCD films (contact angle < 20°) is promising for adhesion and growth of osteoblasts. Besides, the O-terminated surface also seems to support the osteogenic differentiation of the cultivated cells, production of extracellular matrix proteins and subsequent extracellular matrix mineralization.

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THE ROLE OF NITRIC OXIDE ON MINERALIZATION CAPABILITY OF OSTEOBLASTS SEEDED IN A TYPE-I COLLAGEN SCAFFOLD
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In bone, osteoblast-cadherin and connexin-43 gap junctions are important regulators of osteoblast differentiation [1]. Our group works with a bone tissue engineered scaffold consisting of murine embryonic stem cells seeded in a type-I collagen scaffold, and stimulated towards osteoblast differentiation using beta-glycerophosphate (BGP). This cell preparation has been previously shown to successfully drive osteoblast differentiation without ascorbic acid or vitamin D supplementation, and reduces teratoma formation after transplantation in vivo [2]. For this reason, we have investigated the role of communication structures and confined compression on the ability of osteoblasts to initiate mineralization within the scaffold, and what effect inhibiting cell communication has on cell function. From these previous studies, we found that if mechanical loading is applied to cells at an early stage of differentiation (Day 5), the mineralization present downstream is much greater and is more structured. If cell communication is inhibited, osteoblast differentiation is impaired, and cells are unable to recover their function even after communication is restored. Evidence from gene knock-out studies have shown that bone formation is in part mediated by nitric oxide [3]. To build on our understanding of the micro-scale environment in this scaffold, we investigated the extent to which nitric oxide is produced under loading, and whether applying an external nitric oxide donor to cells could be used as a scalable factor to optimize this scaffold for bone healing applications. Murine embryonic stem cells were maintained in T75 culture flasks, and after 3 to 4 passages, 1 million cells were spun down and resuspended in media containing BGP (260mg/mL media). The cells were combined with purified bovine collagen-I (Advanced BioMatrix, 800ul collagen/1ml gel), seeded in 24-well plates, and incubated at 37°C for 5, 15, 20, and 30 days. At each time point, free nitric oxide production and nitric oxide synthase activity was detected with DAF-2DA (4mM) fluorescent staining. DAF-2DA staining was also performed in the presence of octanol (general communication blocker), and 18-α-glycyrrhetinic acid (AGA, a specific gap junction blocker). 5% confined compression of constructs was performed using a custom FlexCell loading plate with a loading regime of 4 hours/day for two days, frequency of 1Hz, and a strain rate of 1%/second. S-nitroso-N-acetyl-penicillamine (SNAP, 100mM) was used as a nitric oxide donor. von Kossa staining for mineralization in the presence and absence of nitric oxide donor, loading, and communication inhibitors was performed. RT-PCR for osteoblast differentiation markers, osteoblast-cadherin, connexin-43, and nitric oxide synthases 1-3 were measured for all treatment groups. Results from this study showed that nitric oxide is actively produced by cells within this scaffold and increases as differentiation progresses. Further, nitric oxide production increases when cells are loaded. This may be attributed to upregulation of connexin-43 when cells are loaded. Application of SNAP increased mineralization present in the matrix in Day 5 constructs, and helped to recover the initiation of mineralization in constructs where communication had been inhibited. Gene expression showed that endothelial nitric oxide synthase (NOS3) is the major enzyme involved when cells differentiate and are loaded. SNAP enhanced osteoblast differentiation in both unloaded and loaded conditions, and enhanced Sox2 (osteoblast self-renewal) and Runx2 (early osteoblast marker) expression in constructs treated with communication inhibitors. Therefore, the results of this study indicate that nitric oxide plays a major role in regulating early differentiated osteoblasts to initiate mineralization within this scaffold. Further, SNAP can be used as an exogenous factor to enhance mineralization and can refocus osteoblast differentiation and rescue osteoblast function when communication has been impaired. Thus, we present a scaffold preparation that is reproducible, responsive to loading, permeable to communication inhibitors, maintains cell viability, and allows for the application of a nitric oxide donor to optimize this scaffold for bone healing applications.


DECELLULARIZED SKELETAL MUSCLE SCAFFOLD AS A SUITABLE PRO-MYOGENIC ENVIRONMENT

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Current development in tissue engineering is focused to prepare tissues or organs, which can replace or substitute damaged or missing part of human organism, but artificial matrices still doesn´t satisfy original composition of tissue. A biological scaffold from extracellular matrix can be produced by a variety of decellularization methods whose caveat consists in efficiently eliminating cells from the treated tissue. Our whole-organ decellularization protocol includes osmotic shock which promotes cell lysis followed with immersion with ionic detergent (1% SDS). Residual chemicals agents were washed out with PBS. Efficiency of decellularized protocol was determined by biochemical and histological methods. Western blot proved laminin preservation which is main basement structure in extracellular matrix. Collagen content was measured with hydroxyproline assay. The final concentration of hydroxyproline in decellularized tissue was higher compared to untreated muscle tissue. This difference reflects removal of other protein components. Histological sections were stained with standard haematoxylin-eosin revealing nuclei absence compared to physiological skeletal muscle tissue. Immunostaining of two extracellular matrix proteins (decorin and laminin) indicate that structural and basement membrane component of extracellular matrix are retained. Adhesion and viability of the scaffold was confirmed by re-seeding with C2C12 murine myoblasts. Cell morphology was examined in paraffin-embedded sections at 3, 6 and 9 days after cultivation with myoblasts.

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AUTOLOGOUS CHONDROBLASTS AND ADIPOSE TISSUE-DERIVED STEM CELLS FROM MICROTIA PATIENTS FOR CARTILAGE REGENERATION

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Cartilage has limited regenerative capability, hence its repair in patients with congenital malformations, or following trauma, poses a major challenge for reconstructive surgery. Microtia is a congenital craniofacial deformity in which patients have small, abnormally shaped ears. It is usually accompanied by a narrow, blocked or absent ear canal and consequently hearing loss. It affects 0.4-4.2/10000 births (depending on populations) and it is more common in men. Currently, the most used approach to treat microtia is rib cartilage graft reconstruction; this requires 3-4 surgical stages and is associated with
morbidity at the donor site. Successful development of autologous tissue-engineered cartilage will reduce surgeries and morbidity, and circumvent the need for immunosuppression to avoid rejection.

The aim of this study is to investigate whether chondroblasts, derived from paediatric microtia patient hypomorphic ears, which are normally discharged after surgery, have normal growth and differentiation potential and could therefore provide an appropriate cell source for autologous cell therapy. To this purpose, we have analysed tissue structure, gene expression and the behaviour of cultured cells from ear and rib cartilage biopsies from microtic children who had undergone rib graft reconstruction. Histological analysis shows that whereas the macroscopic organisation of the tissue appears largely lost in microtic ears, with islands of cartilage interspersed in fat tissue, the microscopic structure of the elastic auricular cartilage is maintained. We also show that chondroblasts, as indicated by protein and gene expression analysis, can be obtained and expanded from explants of microtic ears. As indicated by Alcian Blue staining, chondroblasts derived from microtic ears are able to differentiate into cartilage. Comparison of chondrogenic differentiation of microtic chondroblasts and adipose tissue-derived stem cells (ADSCs) after 3 weeks differentiation shows an higher increase in Alcian Blue staining in microtic chondroblasts (17-fold increase in stimulated cells compared to unstimulated cells) than in ADSCs (3-fold increase).

Taken together these initial findings suggest that microtic chondroblasts could provide an useful cells source for ear reconstruction that deserves further characterization.

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