Abstracts List

**TS01**

**PCL nanofibers with magnetic nanoparticles for mesenchymal stem cell proliferation**

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Nowadays magnetic nanoparticles have a great potential for biomedical applications due to their specific properties. These particles usually consist of magnetic component, often magnetite (Fe₃O₄) or other iron oxide, and the nonmagnetic component which ensure interaction with biological material. Magnetic particles have an important role especially for the separation of biomolecules and diagnostics, however in recent years, there are also applications of magnetic nanoparticles in the field of tissue engineering. Mesenchymal stem cells are unique especially for their ability to differentiate into many cell types and are thus very promising tool for tissue regeneration. However, there are problems with their insufficient quantity, so it is necessary to expand these cells in vitro. Herein, nanofibers were created by electrospinning from a mixture of polycaprolactone and magnetic particles (Fe₃O₄) at size of 50 nm. Viability and proliferation of the cells were monitored at 7th and 21st day after seeding. Adhesion and proliferation of the cells were also verified by confocal microscopy. Significantly better viability and proliferation of cells in the presence of magnetic nanoparticles were also increased in case of presence of magnetic nanoparticles in the scaffold. This opens an interesting possibility of mesenchymal stem cell differentiation using magnetic particles. This new method of using magnetic nanoparticles can significantly contribute to the development of tissue engineering techniques and also has the potential to be used as a new technique for efficient expansion of mesenchymal stem cells in vitro for clinical applications.

**TS02**

**Plasmatic modification of PVA nanofibers to enhance adhesion and proliferation of mesenchymal stem cell**

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Polyvinyl alcohol (PVA) nanofibers prepared by method of electrospinning have desirable properties for tissue engineering applications. PVA is a non-toxic, biodegradable and biocompatible polymer, suitable for biomedical engineering. However the usage of PVA is limited because of the -OH functional groups, which are responsible for the solubility of PVA in water. This solubility could be modified with crosslinking techniques. Another challenging problem is the high hydrophilicity of the surface. Therefore, we have developed a coating system of these nanofibers using cold plasma, which modifies the side groups of PVA and allows higher cell adhesion.

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**TS03**

**Polypropylene mesh functionalized by nanofibers and growth factors for incisional hernia regeneration**

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**Aims:** The aim of this study was to develop functionalized scaffold for incisional hernia regeneration. New composite scaffolds, based on polypropylene chirurgical mesh (PP), polycaprolactone (PCL) nanofibers and adhered growth factors (GF) have been prepared. Functionalized scaffold was tested in vitro using 3T3 fibroblasts and subsequently on a rabbit model.

**Materials and methods:** Biocompatibility of the scaffold and its capacity to promote proliferation of the cells was proved in vitro by confocal microscopy and MTT assay. Cell proliferation and viability were evaluated on the day 1, 3, 7, 10, and 14 by MTT assay and live/dead staining (BCECF-AM and Propidium iodide) with subsequent confocal microscopy visualization. The functionalized mesh was implanted into 27 rabbits, divided into six groups. In each animal 5 cm long midline incision was made in the fascia as an incisional hernia model. In Group 1 (the control group) the tissue defect in the fascia was primarily closed using 4/0 PP suture. Group 2 was treated with PP mesh only, while Group 3 was treated with PP mesh functionalized with PCL nanofibers enriched by GF and Group 5 with PP mesh functionalized with PCL nanofibers without GF. Groups 4 and 6 were treated with PCL nanofibers only, with GF (Group 4) or without them (Group 6). 6 week after euthanasia the abdominal area was evaluated by histological analysis and biomechanical testing.

**Results:** In vitro tests confirm improvement on adhesion, proliferation and viability of the cells seeded into scaffold based on PCL nanofibers with adhered GF, comparing with scaffolds base only on PP mesh. Surprisingly analysis of biomechanical tests shows the highest values of maximal strength force in samples based only on PCL nanofibers, although PCL nanofibers show very poor tensile strength alone.

**Conclusion:** Polycaprolactone in form of nanofibers appears to be proper material for incisional hernia reparation. It is the first use of the material in this application. This material will be tested and has a good potential to be clinically used.

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**TS04**

**Advanced nanofibers for regulated drug delivery**

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Monolithic nanofibers are classically used as scaffolding fibrous matrix. Progress in nanofiber research is focused on utilizing nanofibers as carriers for regulated drug delivery.

Our team is developing advanced nanofiber solutions with different type of functionalization. First, we want to present coaxial nanofibers with embedded liposomes as a protecting agent stabilizing encapsulated proteins. The second system is based on coaxial nanofibers with embedded alpha granules as a natural source of growth factors. The third system is based on core/shell nanofibers based on emulsion electrospinning with growth factors. The systems were compared from the view of preservation of enzymatic activity, release behaviour and effect on mesenchymal stem cells proliferation and differentiation. The work brings new insight into comparison of coaxial and emulsion electrospinning.
**TS05**

**Influence of electrohydrodynamic jet (EHJD) printing on the cell behavior**

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Objectives: Organ printing (OP) as new direction of tissue engineering tends to fabricate natural-like functional biological constructs for regeneration of damaged organs, as well as for in vitro studies. OP is a multi-step complex process that is composed of three sequential steps: “blueprints” preparation, cell encapsulation and organ maturation [1]. Cell encapsulation as an important step of OP was well studied in the past decade and were developed several protocols in that field, as well as improvements in printing apparatus [2]. However, it’s also very important to evaluate the influence of cell encapsulation process on cell behavior. This study provides preliminary data on the encapsulated cells behavior.

Materials and methods: SHSY5Y human neuroblastoma cell line was used as model. Cells were encapsulated by the EHDJ method in 2% alginate under 8 kV in 200 mM beads, and studied for viability, activity and proliferation through calcein/propidium iodide staining with following confocal visualization; DNA quantification assay accordingly. Expression of heat shock protein (HSP70B), apoptotic executor (CASP3), necrotic (HMGB1) and hypoxic (HYOU1) markers, and cell adhesion molecules (CDH2) were evaluated through RT-qPCR assay.

Results: Live/Dead assay shows cells stay alive during the whole experimental time (7 days), however undergo proliferation down-regulation (PicoGreen DNA quantification assay). RT-qPCR shows 20-60 times increase in HSP70B expression. Double overexpression of caspese-3 gene was detected in first days, and followed by it’s expression stabilization. There was not found significant differences in necrosis marker (HMGB1) expression, neither in hypoxia marker (HYOU1). Double up-regulation of adherence molecule expression (cadherin-2) was detected in first days and followed by significant down-regulation of cadherin-2.

Conclusions: The process of EHDJ encapsulation was found has no cytotoxic effect on cells viability. However, it leads to cell stress accumulation, and induces HSP70B overexpression, that in turn induces cell stress resisting and cells recovering. In fact, it was seen the absence of stress markers expression—apoptotic, necrotic and hypoxic, according to the literature data. Under HSP70B overexpressed conditions different types of cells are protected against stresses such as heat, apoptotic markers or hypoxia [3].


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**TS06**

**Osteogenic differentiation of rabbit bone marrow mesenchymal stem cells in silk fibroin loaded hydroxyapatite/PLGA scaffold**

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Silk fibroin can be used as artificial bone materials due to the excellent mechanical properties and cell compatibility. However, silk fibroin have been studied type of hybrid silk scaffold with the addition of various materials because its vulnerability. Therefore, we prepared hydroxyapatite/PLGA scaffolds loaded various ratio of silk fibroin to complement the disadvantage of the silk fibroin. To investigate their possibility as artificial bone, silk fibroin loaded hydroxyapatite/PLGA scaffolds were experimented with diverse methods. The physical properties of the scaffold were confirmed by the compressive strength, DSC, FT-IR, SEM. To analyze the level of osteogenic differentiation at each time point after cell seeding, ALP activity assay, biological assay with osteocalcin, collagen type I and Runx2, histological analysis were carried out. The results demonstrated that silk fibroin content affected on osteogenic differentiation and 80% of silk fibroin added hydroxyapatite/PLGA scaffolds has better effect than other scaffolds. Thus, this study suggests that 80% silk fibroin loaded hydroxyapatite/PLGA scaffold should serve as structural bases for tissue engineered bone and help osteogenic differentiation.

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**TS07**

The fabrication of silk film for adhesion and proliferation of corneal endothelial cells

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Silk extracted from the cocoons has been used as a biomaterials for tissue engineering applications. These silk fibers are composed of fibroin and sericin surrounding fibroin. While the manufacturing silk film, sericin is normally removed during the degumming process because sericin is known to form fragile films. However, the extraction method to remove sericin completely is too harsh condition on protein denature. Therefore, by varying time, temperature and concentration of Na2CO3 during degumming process, we prepared silk films with various concentrations of sericin. Among the produced films, we chose film having suitable properties and cultivated corneal endothelial cells (CECs) on prepared silk films. To examine characterization of silk films, we tested several analyses. Mechanical property through tensile strength, FTIR spectra, DSC measurement, water contact angle, in vitro degradation and transparency were performed. After physical properties of silk films were confirmed, then, CECs were seeded on silk films and examined the influence on adhesion and proliferation of CECs on silk films. We measured the cell adhesion, cell viability, morphology and specific mRNA expression. Through this study, we confirmed that harsh treatment during degumming process generated silk films having worse physical property. The adhesion and proliferation of CECs were good on silk film with better physical property. Biomaterials for corneal tissue engineering should meet several features for potential utility including transparency, mechanical property and biodegradation. Silk film as a biomaterial for cornea that we made was designed and characterized to satisfy these functional requirements. Therefore, we concluded that the silk film developed in this study has possibility of applications for future use on CEC.

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**TS08**

The effect of hydroxyapatite on osteogenic differentiation of bone marrow stem cell

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Hydroxyapatite (HAp) has biocompatibility and bioactivity and similar to bone of in human body. The purpose of this study is to evaluate osteogenic differentiation of bone marrow stem cell (BMSC) in PLGA Scaffold added various ratio (0%, 10%, 20%, 40% and 60%) of hydroxyapatite. PLGA and PLGA/HAp scaffold were prepared using solvent casting/salt-leaching method. BMSC was seeded on the PLGA and PLGA/HAp scaffold and the samples were cultured in 37 incubator with 5% CO2 for 28 days. Alkaline phosphatase (ALP) was carried out to evaluate alkaline phosphatase activity for 1, 3, 7, 10 and 14 days. Alizarin red stating was performed to identify calcium in scaffold for 1, 7, 14, 21 and 28 days. Compressive strength was measured to evaluate mechanical property of scaffold. To confirm cell viability, MTT was carried out for 1, 3, 7, 14 and 28 days. RT-PCR was performed to verify specific marker expression of osteoblast and stem cell for 7, 14, 21 and 28 days. In the study, HAp may help osteogenic differentiation of BMSC.

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Effect of osteogenic differentiation of chondrocyte in PLGA/hydroxyapatite/silk scaffolds for bone tissue engineering

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Bone is a highly complex tissue which provides mechanical support, and acts as mineral reservoir. Poly(lactic-co-glycolic acid)(PLGA) has several advantages such as good biocompatibility, easy of processing and good physical stability. But, the hydrophobic surface of PLGA is not conducive to cell adhesion. So, we used the silk as a natural biomaterial, which it could be improved by impregnated into PLGA. However, as silk is not an osteogenic material, hydroxyapatite (HAp) with osteoconductive property was added. In the study, PLGA scaffold as a control group, 10 wt% HAp/PLGA, 10 wt% Silk/PLGA, 5 wt% HAp 5 wt% Silk/PLGA and 10 wt% Silk/PLGA scaffolds as experiment groups were prepared. When chondrocyte was seeded on the scaffold, we identified the effect of scaffold for bone regeneration. Cell viability by MTT, adhesion and proliferation by SEM were evaluated. Also, the degree of differentiation of bone by ALP assay and specific gene expression by RT-PCR were analyzed. 10 wt% HAp/10 wt% Silk/PLGA scaffold than any other scaffold in the analysis of ALP was significantly increased over time. And the result of RT-PCR showed that the expression of osteocalcin and type I collagen in 10 wt% HAp/10 wt% Silk/PLGA scaffolds increased with the increase of culture time. These results suggest that 10 wt% HAp/10 wt% Silk/PLGA scaffold should serve as structural bases for bone tissue engineering.

This research was supported by WCU (R31-20029) and Bio-industry Technology Development Program (112007-05-1-SB010), Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.
New treatment formulations for skin regeneration and wound infections have recently been the focus of research in the biomedical field, as they are one of the most common healthcare-associated infections. Antimicrobial peptides (AMPs) are a class of small molecules that can be used in the treatment of skin and wound infections as they occur as part of the innate defense mechanism in many organisms, even in microbes and virus, displaying immunomodulatory effects. With advances in protein engineering and recombinant DNA technology, it is now possible to reengineer protein-based materials with added functionality. Indeed, recombinant DNA technology allows combining in the same molecule distinct functionalities, leading to the production of a chimeric protein displaying the properties of each block of amino acids. With the aim of developing novel advanced materials and ultimately, the fabrication of advanced medical devices, hereby we describe the development, processing and characterization of a new recombinant protein-based polymer (rPBP) with antimicrobial activity. The functional rPBP comprises a functional domain based on a synthetic cationic AMP, fused in frame with an elastin-like-polymer consisting of 200 repeats of VPAYV (A200), as structural unit.

The functional polymer, processed into free standing films by solvent cast, was analyzed for its secondary structure by circular dichroism and FT-IR and tested for their antimicrobial activity against different bacterial and fungal species, in both ex vivo and in vitro conditions. For ex vivo conditions, a new method was developed using pig skin as a model. When in contact with the infected skin, the functionalized polymer showed a good inhibition against the different microorganisms tested after 3 h of contact with skin, indicating that AMP:A200 films are promising candidates for application in skin treatment and wound infections. In addition, in vitro antimicrobial assays demonstrated that the chimeric AMP:A200 polymer is a potent antimicrobial material against a wide range of bacterial species, both gram positive and negative, as well as against yeast species. The antimicrobial activity was dependent on the time of exposition and remarkably, in some cases, almost 100% of microbial cell death was detected after 30 minutes contact with the cast films. Furthermore, the immunomodulatory effects of the AMP:A200 for wound healing and are currently underway.

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**TS13**

**Soft-matrices based on fibrous proteins for biofabrication**

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Printing various living cells within 3D constructs in defined locations next to each other is a relatively new approach in tissue engineering. Alginate (Alg) is a natural, biocompatible polysaccharide that forms a stable hydrogel. However, it is an inert material and does not promote cell attachment. Herein, our aim is to introduce new hybrid hydrogels to control spatial arrangements of multiple cell types in defined geometry, by blending alginate with different fibrous proteins, which form homogeneous secondary structures via self-assembly. Thus, in this work the development of novel 2D matrices for further constructions of 3D architectures is presented, aiming to address specific issues in biofabrication. Different fibrous proteins, such as silk fibrin from Bombyx mori, were previously extracted and characterized in terms of protein content (Lowry and SDS-Page). To obtain the hydrogels in 2D and 3D configurations, Alg (2%, w/v) was mixed with fibrous proteins (2%, w/v) to prepare blends of 50/50. To study the swelling ratio and the weight loss, the blends were immersed in HBSS and DMEM, over 21 days, and it was founded that these two parameters are dependent on the composition. The interaction between Alg and different fibrous proteins was further investigated using thermal analysis (DSC) and FTIR assessments. The results demonstrated changes in the protein conformation after blending. Dynamic mechanical thermal analysis (DMTA) on 2D films revealed viscoelastic behavior. Moreover, the cell-material interaction of the hybrid materials was conducted using Human endothelial and fibroblast cells. Together with SEM, viability and live/dead staining measurements showed no cytotoxic effect and the spread of cells in all Alg/protein hydrogels. Therefore, it can be concluded that the synergistic effects of each blend component allow the improvement of the properties of the material used to prepare the 2D and 3D geometries. Furthermore, the gel-network and mechanical properties were assessed and tuned by analyzing the response of different cell types to obtain an ideal hydrogel system suitable for biofabrication.

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**TS14**

**Electrospun recombinant protein-based polymers fibre matrices for skin regeneration**

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Protein-based-polymers (PBPs) are abundant macromolecules present in a wide variety of organisms fulfilling structural and mechanical roles. Due to its remarkable mechanical properties but also their improved biocompatibility, PBPs have drawn much attention in the development of nanostructured scaffolds for tissue engineering applications. With the advance of protein engineering and nano(bio)technologies, and by using recombinant DNA technology, it is now possible to design and construct recombinant PBPs (rPBPs) with an absolute control of its composition, sequence and length. Silk-elastin-like proteins (SELPs) are a class of rPBPs composed of silk and elastin repeating units. In the present work, we report the electrospinning of SELP-59-A, a new co-polymer with formulation (SS69)9, where S corresponds to the number of repetitions of the silk block (GAGAGS) and E to the number of elastin blocks (VPAVG). Electrospinning was performed using aqueous or formic acid solutions at different SELP-59-A concentrations without adding external agents. Polymer concentration and solvent were showed to play a crucial role in the process of electrospinning. While concentrations of polymer solution led to the formation of nano/microsized structures, higher polymer concentrations produced fibers with increasing diameter and size distribution. Comparing the solvents, the electrospin fibers obtained from aqueous solution displayed higher diameter and size distribution. As the electrospun membranes are highly water soluble, structure stabilization and water insolubility were rendered by treatment with methanol. Changes in the secondary structure were analyzed by FTIR showing that methanol induces a time-dependent conversion of random coils into beta-sheets. The methanol-treated electrospun scaffolds were further characterized in terms of its wettability, displaying a water contact angle of 69°, a degree of swelling in the range of 570-720% and water vapor transmission rate of 1083 g/m2/day. The mechanical properties measured by uniaxial stress-strain analysis revealed an average modulus of elasticity of ~126 MPa. Furthermore, the produced electrospin SELP-59-A fiber matrices demonstrated to be non cytotoxic and able to support adhesion and proliferation of normal human skin fibroblasts (BJ-Sta cell line). These properties indicate that SELP-59-A scaffolds have potential to be applied as wound dressings for skin regeneration purposes, especially considering moderate exuding wounds.

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Meniscus is a fibrocartilaginous tissue that has an important role in biomechanics of the knee joint. Fibrochondrocytes and fibroblast-like cells are the two main cell populations present in the meniscus. Meniscus is distinguished into two regions: avascular and vascular region. Cellularity varies within the human meniscus, specifically between avascular and vascular regions of the meniscus, but also between anterior, medial and posterior parts. Cellularity is one of the important characteristics that should be considered in tissue engineering and regenerative medicine strategies. The aim of this study is to calculate the 3D cell density of human meniscus using histological slides. Meniscus tissues obtained from donors are prepared into Giemsa stained histological slices with a thickness of 30 μm. Slices are grouped by their anatomical location into three parts: anterior, medial and posterior. Cells in the defined areas of avascular and vascular regions are counted either as fibrochondrocytes or as non-fibrochondrocytes using a stereomicroscope. 3D cell densities of different region and parts of the meniscus are estimated by calculating the number of the cells found in unit volume. The initial results show that the 3D cell density is around 8000 cells/mm³ in vascular part, that is the almost double of the density in avascular part. Chondrocytes show that the 3D cell density is around 8000 cells/mm³ in vascular part and less than the half in the vascular part. This work aims to contribute to the knowledge of cellularity of human meniscus and facilitates the development of more efficient strategies for meniscus tissue engineering. The authors thank the financial support of the MultiScale-Human project (Contract number: MRTN-CT-2011-289897) in the Marie Curie Actions—Initial Training Networks.

Tissue engineering is an emerging field focused on the development of novel bioactive multifunctional materials that can be used to replace damaged and failing tissues. However, these biomaterials often present several problems such as loss of mechanical and/or biological properties and adverse immune responses. The use of natural polymers, such as proteins, provides a promising solution for these drawbacks. With advances in recombinant DNA technology and biotechnology, it is possible to design and produce new materials with different features by combining domains of different proteins in the same fusion protein. Spider dragline silk proteins have been suggested to have a large potential for many different biomedical applications due to its outstanding mechanical properties. In addition, spider silk is also biocompatible, hypoallergenic and completely biodegradable. Recently, silk copolymers based on repeats of the consensus sequence of MaSp1 (major ampullate spidroin I) from Nephila clavipes (6mer) have been fused with different functional proteins, peptides and protein motifs, showing promising results [1, 2]. In this project, by exploring the use of recombinant DNA techniques, we have constructed new silk copolymers composed of a structural motif (6mer) fused with functional domains namely GFOGER (from collagen type I) and FNII (fibronectin domain II); both are involved in cell adhesion and angiogenesis processes which are key factors in tissue engineering. Expression and purification of the new chimeric proteins were successfully attained in Escherichia coli by means of auto-induction media. Furthermore, formic acid can be explored as a solvent for processing of the aforementioned recombinant copolymers. Results from the characterization of these biomaterials will be presented.
Injectable and dual-stimuli-responsive silk fibroin hydrogels for tissue engineering and regenerative medicine applications

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Hydrogels have been attracting increasing attentions, since they mimic the extracellular matrix environment and can be used as delivery systems for cells or bioactive agents. Silk fibroin is a well recognized and compatible biopolymer for tissue engineering applications. The traditional methods to prepare silk fibroin (SF) hydrogels take advantage of the conformation transition from amorphous to β-sheet in aqueous SF solution. During this procedure, the gelation time normally varies from hours to months depending on the methods used. The relative long gelation time of those methods limits their practicality as in situ injectable systems for incorporation of cells or drugs. Furthermore, most of these approaches are not suitable for cell/drug incorporation in the silk hydrogel. The current study provides an approach to develop a new SF hydrogel within a few minutes in physiological conditions via peroxidase mediated cross-linking. The influence of silk concentration, and the content of peroxidase and hydrogen peroxide on the physicochemical properties of the hydrogels were studied. The results showed that the gelation time of the silk hydrogel decreased with increasing silk and peroxidase content, and can be tuned between 4 to 50 minutes. The storage moduli of the hydrogels improved via increasing the hydrogen peroxide content and silk concentration, ranging from 0.25 to 5.20 kPa. The fast formed hydrogels showed extreme elasticity and transparent appearance. There were no differences of the silk hydrogel in the visible light absorbance, before and after the gelation. The dominant conformation of the formed silk hydrogels was amorphous, confirmed by Fourier Transform Infrared Spectroscopy. Interestingly, the prepared hydrogels were of ionic strength and pH responsive properties. Its size or wet weight increased in solutions of low ionic strength or basic pH, and vice versa. Cells could be incorporated into the hydrogels and were viable up to 11 days. Cytotoxicity results demonstrated that these hydrogels were non-cytotoxic. After subcutaneous implantation in mice for 2 and 4 weeks, the SF hydrogels induced no inflammation reactions in vivo. This study provides a facile approach to prepare injectable SF hydrogels with dual stimuli-responsive properties. The unique properties of these hydrogels exhibit innumerable potential applications, such as for oral drug delivery, adipose tissue regeneration, artificial cornea, wound dressing, and cartilage regeneration.

Reference:
Le-Ping Yan, Ana L. Oliveira, Joaquim M. Oliveira, Diana Ribeiro Pereira, Raúl A. Sousa, Rui L. Reis. Hydrogels derived from silk fibroin: Methods and uses thereof. National Patent Nr. 106041, priority date: 06-12, 2011.

Chitosan nanofibers as scaffolds for peripheral nerve regeneration

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Damage to the Peripheral Nervous System (PNS) is remarkably common and occurs mainly from trauma or a complication of surgery. Although recovery of nerve function occurs in some mild injuries, outcomes are frequently poor following severe trauma, resulting in long-term impairment of limb function, dysthesia and pain, often with associated psychological effects. In contrast to the central nervous system, the PNS includes an intrinsic capacity to regenerate. Currently, the gold standard autograft repair of the damaged peripheral nerve is far from optimal and is often disappointing. The alternative to the use of grafts is the use of a scaffold that consists in an artificial nerve guide, namely a hollow tube, combined with engineered biomaterials filling its interior in order to provide topographical cues as it has been postulated that axon elongation requires guidance by contact with appropriate substrates. In the scope of the Biohybrid project, chitosan (CS) powders from Altakitin were supplied and its cytotoxicity was assessed. According to cell viability percentage, no cytotoxicity was observed. Using these biomedical grade powders, biodegradable scaffolds were developed to support neuronal regeneration using the electrospinning technique to produce nanofibers from chitosan solutions. Random and aligned nanofibers were produced and characterized, using techniques such as FTIR, SEM, AFM, DSC and contact angle. To produce a mesh of random chitosan fibers with no beads or other defects, a 5% chitosan solution in trifluoroacetic-acid and dichloromethane as solvents, in the proportion of 70:30 was used. They are super hydrophilic, defect-free fibers with no beads or other defects, a 5% chitosan solution in trifluoroacetic-acid and dichloromethane as solvents, in the proportion of 70:30 was used. They are super hydrophilic, defect-free and have an average diameter of 184 ± 36 nm. Yarns of aligned chitosan nanofibers were obtained using the two blades placed in line set up, with the exact same conditions used to obtain random nanofibers. In vitro tests to characterize L929 cells viability (MTS assay) and proliferation (DNA quantification) on nanofibers scaffold were performed.
Biological characterization of rabbit nucleus pulposus cells on a biphasic scaffold made of polycaprolactone and methacrylated gellan gum hydrogel

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Intervertebral disc (IVD) degeneration (IDD) is nowadays considered as the main physiological cause for low back pain (LBP). LBP is known to affect people of any age, having a world socioeconomic burden of 70 billion euros per year[1]. Current LBP treatments only treat the symptoms without solving the problem. So, finding new ways of treating IDD is finding new ways of reducing socioeconomic impact created by LBP. Tissue Engineering (TE) is an exponentially growing area due to its potential of finding patient-specific treatments in terms of immunological compatibility by using patient’s own cells. Though, it is time for TE to take a step towards an even more patient-specific way of treating diseases. Reverse Engineering (RE) appeared as a way to find how a system works without having its blueprints. RE combined with 3D printing can help researchers reproduce any kind of anatomical structure. So, by combining both TE and RE it is possible to develop not only patient-specific treatment strategy in terms of immunological compatibility but also in terms of structure. The IVDs, which are located on the spine, are composed by a hydrogel-like nucleus pulposus (NP) core that is contained vertically by cartilaginous end-plates, and horizontally by fibrocartilage ring called annulus fibrosus (AF). The purpose of this study is to use Reverse and Tissue Engineering to develop custom-made implants for IVD regeneration. Rabbit spines were analyzed by micro-computerized tomography and were RE into a virtual 3D model which was then 3D printed with polycaprolactone, that has already shown, in the literature, a great potential as a material to develop AF scaffolds[2]. The solid scaffold was then filled with rabbit NP cell-laden methacrylated gellan gum (GG-MA) hydrogel. The GG-MA hydrogel has been shown great promise for NP regeneration, in vitro and in vivo[3]. This way, a fully patient-specific biphasic scaffold was produced which mimics the native IVD’s structure and biomechanics.

References:

Development of novel bilayered structures to be used as an osteochondral in vitro model

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Proper in vitro models to evaluate the performance of relevant tissue engineered constructs are still a major demand of the field. The use of in vitro platforms in research presents obvious ethical and cost advantages over in vivo models. In vitro models can also offer important scientific advantages, as for example, the study of biological mechanisms of action because it is easier to isolate an experimental variable and measure its impact on a simple, well controlled system. The aim of this project is to create an osteochondral in vitro model. As proof-of-concept, two different bilayered sponge-like scaffolds were developed to act as a template for co-culturing rabbit adipose stem cells (ASCs)-derived osteoblasts and chondrocytes. Bilayered low acyl gellan gum (LAGG)-LAGG/hydroxyapatite (HAp) spongy structures with and without Gelatin were produced respectively integrating cartilage- and bone-like layers. The freeze-dried bilayered scaffolds composed by LAGG2%(w/v)-LAGG2%/HAp30% (w/w) and LAGG/Gelatin 1:1 2%(w/v)-LAGG/Gelatin 1:1 2%/HAp30% (w/w) have a gradient of HAp in the bone-like layer that, unlike cartilage-like layer, present a bioactive behavior. The bilayered structures possess about 90% porosity, 500 μm of pore size and 85% interconnectivity as determined by Micro-CT analysis. Swelling and degradation tests revealed that the structures can absorb about 120% of their weight and lost 10% of their mass after 30 days in phosphate buffered saline solution. In vitro studies with rASCs from Fat Pad (knee) are being performed to study cell adhesion and proliferation. A rotational dual chamber bioreactor was fabricated in-house to improve medium diffusion into the structures, to allow the use of two different culture mediums for each layer, to homogenize the cell distribution in the scaffolds, as well as to introduce mechanical stimuli by 180º stirring and compression of the top layer. So far, the results have shown that the developed bilayered scaffolds have a great potential for finding application as a screening platform of new therapeutic approaches for the treatment of osteochondral tissue disorders.

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In drug delivery there has been great interest in the development of nano- and microcarriers of active agents to control their release kinetics and their efficacy by delivering them to where they are most needed. A possible method of delivery is that of carrier internalization by cells. By delivering an active agent directly to the cells, high drug concentrations can be made available to the target cells while circumventing undesirable side effects to surrounding tissues due to premature drug leakage. In this work, microcapsules prepared using layer-by-layer were conceived using chitosan and biomimetic elastin-like recombinamers (ELRs) as constituents assembled onto templates of calcium carbonate microparticles. Two types of ELRs were used: one containing the bioactive aminoacid sequence RGD and the other a scrambled nonfunctional RDG. Scanning electron microscopy (SEM) showed no morphological differences among both types of microcapsules, being spherical with around 4 μm in diameter. Cell viability studies were performed using human mesenchymal stem cells (hMSCs) and microcapsule/cell ratios from 5:1 to 100:1. After 3 and 72 h of incubation, no significant cytotoxicity was observed in respect to a positive control of hMSCs co-incubated with microcapsules. The cells were kept in culture for another 7 days in absence of microcapsules. Live/dead assays confirmed that cells retained their cellular integrity, thus the contact of hMSCs with either functionalized microcapsule type does not result in cellular death. The internalization efficacy of microcapsules was assessed by flow cytometry and microscopy analysis. To our knowledge, this is the first time that the internalization effectiveness of RGD-functionalized LbL microcapsules is compared with a nonfunctional analogue microcapsule type. Loading them with DQ-ovalbumin permitted to follow the intracellular traffic and degradation by monitoring fluorescence changes. The data indicated that 63% of the hMSCs have internalized theRGD-functionalized microcapsules, while their nonfunctional analogue triggered internalization in around 53% of the cells. No statistical differences were found between both cases, suggesting that macropinocytosis should be the major endocytosis mechanism involved in the cellular uptake of this class of carrier devices and that the exhibition of the RGD/ RDG motifs does not influence significantly the incorporation of the microcapsules by the hMSCs. Intracellular processing was assessed by qualitative fluorescence variations showing that this phenomenon was faster for the RGD-functionalized microcapsules. The developed multilayer microcapsules using biomimetic ingredients for intracellular delivery let foresee new strategies to increase the availability of molecules of interest in cells and for targeted biomedical applications.

Layer-by-layer (LbL) is a mild and versatile surface modification technique that allows producing robust coatings even in substrates with complex geometries. Taking advantage of the 3D possibilities opened by LbL, we present customized compartmentalized capsules inspired by the complex structure of cells, with temperature and magnetic-based responsiveness, and hierarchical organization from the nano- to the visible scales. These capsules consisted in liquefied alginate macroscopic beads coated with a chitosan/alginate shell confining molecular compounds and microcapsules, with the latter encapsulating further either more molecular compounds or magnetic nanoparticles (MNP). The microcapsules were constructed resorting to LbL, assembling chitosan and temperature-responsive elastin-like recombinamers (ELR) around calcium carbonate particles. The appropriate LbL assembly conditions of these ingredients were screened resorting to quartz-crystal microbalance. The multilayer build-up of chitosan and one of nine ELRs differing in aminoacid content, length and biofunctionality was followed in situ at pH 4.0 and 5.5, accounting for 18 combinatorial conditions. Their thicknesses were estimated using the Voigt model, revealing that thicker films were obtained in the presence of hydrophobic interactions between ELRs and partially neutralized chitosan[1]. From these results, the microcapsules were constructed with chitosan and a RGD-containing ELR at pH 5.5. Release studies at 25 and 37 °C with BSA demonstrated that the microcapsules are less permeable at physiological temperature, providing a sustained release over 14 days. The microcapsules were also noncytotoxic towards L929 cells[2]. The microcapsules could also be loaded with MNP, which react towards external magnetic fields. Finally, the compartmentalized liquefied macrocapsule was constructed bearing the microcapsules and rhodamine for quick assessment of the multilayer coatings’ permeability. For 25 °C and 37 °C, rhodamine encapsulated within the inner microcapsules showed sustained release, with the diffusion kinetics being even lower at physiological temperature. Rhodamine encapsulated in the outer alginate compartment did not show significant difference between each temperature. The devices were robust and could withstand handling and centrifugal stress. MNP loaded within the microcapsules were able to render the whole compartmentalized device magnetic responsive[3]. Such customizable system can be envisaged to transport bioactive agents and cells in tissue engineering applications and construct disease and microtissue production models.

References:
Regenerative endodontics: revascularization in an immature permanent tooth using platelet rich plasma (PRP) evaluated with dental computed tomography

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Introduction: Regenerative medicine offers several advantages for the treatment of disease with its aim of “replacing or regenerating human cells, tissues or organs to restore or establish normal function”. Considerable recent enthusiasm and effort towards the application of regenerative therapy in endodontics is leading to an exciting future potential for engineering not only pulp tissue but also whole tooth. Regenerative endodontic procedures can be defined as biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex. Various approaches include root-canal revascularization, postnatal (adult) stem cell therapy, pulp implant, scaffold implant, three-dimensional cell printing, injectable scaffolds and gene therapy. This case report presents revascularization of pulp in necrotic immature tooth which is one of the most conservative and practical approaches of this revolutionary therapy.

Discussion: The PRP enabled revascularization of root canal by acting as a biological scaffold. It can cause the sustained release of growth factors, enhancing the recruitment, retention, and proliferation of undifferentiated mesenchymal and endothelial cells from the periapical area. It stimulates collagen production. It also produces anti-inflammatory agents (ANTES/CCL5 [Regulated upon activation, normal T-cell expressed, and secreted, a protein classified as a chemotactic cytokine or chemokine]) that controls the local inflammation and improves soft- and hard-tissue wound healing.

Conclusion: Based on the results of our study, we conclude that tissue regeneration in root canal is possible in necrotic immature tooth using PRP. Replacement of the necrotic pulp by vital tissue is better than replacement with biomaterials, gutta-percha and root canal sealer.

Silk-based 3D biotextiles support human adipose derived stem cells towards osteogenic differentiation

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Textile-based technologies are considered as potential routes for TE applications, since they allow for producing finely tuned fibre-based porous scaffolds with a very reproducible and interconnected intra-architectural geometry, increasing the surface area for cell attachment and tissue ingrowth. Human Adipose-derived Stem Cells (hASCs) constitute an emerging possibility for regenerative medicine and tissue replacement therapies. Their osteogenic differentiation potential, easy isolation, expansion and in vitro proliferation demonstrate their promising prospects in bone regeneration. The present work aims at evaluating the potential of recently developed 3D silk-based biotextile structures to support hASCs adhesion, proliferation and osteogenic differentiation. The 3D spacer structures were processed by using a knitting technology. Two knitted silk layers were assembled and spaced by a monofilament of polyethylene terephthalate (PET). A 3D structure made entirely of PET was also used for comparative purposes. Cells were seeded over the constructs for 7, 14, 21 and 28 days in basal and osteogenic conditions. HASCs adhesion, proliferation and the osteogenic differentiation potential of the textile structures were analysed through Scanning Electron Microscopy (SEM) and preliminary biological assays: alkaline phosphatase (ALP), DNA and Ca²⁺ quantification. The obtained results validate the developed constructs as suitable for hASCs adhesion, proliferation and differentiation into an osteoblastic lineage. Great evidences of extracellular matrix mineralization were observed as well as a deeply cell penetration and colonization into the scaffolds interior. The positive influence of the produced fibre-base architecture on the osteogenic differentiation of hASCs and ECM production validates this technology for being used in bone TE. Moreover, the versatility and reproducibility of this knitting technology can allow for further industrialization of TE products.
Magnetic nanoparticles (MNPs) are of great interest for diverse biomedical applications such as hyperthermia, contrast agent for MRI, magnetic drug delivery, and cell mechanosensitive receptor manipulation to induce cell differentiation and proliferation. They are also potentially useful for cell labeling. However, a field that has not been fully explored is the use of MNPs for cellular based therapies. The therapeutic cells, loaded with MNPs, could be delivered by intravenous injection and be attracted to sites of injury through the application of an external magnetic field. In this work we have studied the magnetite \((\text{Fe}_3\text{O}_4)\) nanoparticles uptake capacities by a L929 fibroblast mouse cell line. Using a nanoparticle library encompassing both spherical and rod-shaped MNPs with diameters between 20 nm and 90 nm, respectively, we have investigated the influence of time and MNPs concentration on cell internalization and viability. The MNPs were prepared via a facile way by co-precipitation reaction, then coated at the surface level with 3-aminopropyl trimethoxysilane by a silanization reaction, and finally labeled with fluorescent molecules (Rhodamine B isothiocyanate and fluorescein-5(6)-isothiocyanate). The successful coating of the MNPs was assessed through ninhydrin assay, and DLS measurements. Cytotoxicity and viability tests were also performed. The internalization efficiency of the MNPs was assessed by measuring the internalized iron content through Inductively Coupled Plasma (ICP). The findings from this study will have implications in the chemical design of nanostructures for cell based therapies.

Some marine species, such as mussels, can strongly attach themselves to rocks in the difficult conditions of the sea. In fact, marine mussels secrete adhesive proteins that show a high adhesion to both inorganic and organic surfaces in aqueous environments. These proteins have an amino acid designated as 3,4-dihydroxy-L-phenylalanine (DOPA) that in turn possesses catechol groups that are primarily responsible for these strong adhesive bonds. Inspired by this behaviour, layer-by-layer (LbL) films based on polymers that contain catechol groups were developed. It is expected that such materials will present an enhanced cell adhesion when they are applied in biomedical applications. Dopamine-modified hyaluronic acid (HA-DN), which possesses catechol groups, was prepared by carbodiimide chemistry. This conjugate was characterized by distinct techniques, such as nuclear magnetic resonance (NMR) and ultra-violet spectrophotometry (UV). Then films were developed based on chitosan (CHI) and HA-DN using the Layer-by-Layer (LbL) technique. The formation of these films was investigated \textit{in-situ} by quartz crystal microbalance with dissipation monitoring (QCM-D). The adhesion properties of the coatings were also analyzed. \textit{In vitro} tests using distinct cell sources revealed an enhanced cell adhesion, proliferation and viability for the films that contain catechol groups, which demonstrates their potential to be used in biomedical applications.
Nanostructured hollow tubes based on chitosan and alginate multilayers

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The design and production of structures with nanometer-sized polymer films based on layer-by-layer (LbL) are of particular interest for tissue engineering since they allow the precise control of physical and biochemical cues, as well as the recreation of the natural complexity of ECM. In this work, we develop a method for the preparation of nanostructured hollow multilayers tubes combining LbL and template leaching. The biocompatible multilayers films were based on the alternate deposition of chitosan and alginate. Our aim was to produce hollow tubes based on polyelectrolyte multilayer films with tuned physico-chemical properties and study their effects on cell behaviour. The deposition of chitosan (CHIT) and alginate (ALG) for the production of multilayers at 2D level was followed by quartz crystal microbalance with dissipation (QCM-D) and the final tubular structure were characterized by differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), scanning electronic microscopy (SEM), water uptake and mechanical tests, including dynamic mechanic analysis (DMA) at physiological conditions. It was found that the physico-chemical properties of these tubes can be tailored by chemical crosslinking with genipin which enhances the mechanical properties of the construct and restrain the high water-uptake of polysaccharides –based polyelectrolytes multilayer film. The water uptake decrease from about 300% to 100% after the crosslinking. On the other side, the mechanical properties confirmed the viscoelastic properties and a storage and loss modulus about two times higher. We further evaluate the biological performance in terms of cell adhesion, viability and proliferation. The results obtained with the crosslinked films have demonstrated that these were more suitable structures for cell adhesion and spreading on polymeric films that are otherwise non-cell adhesive. The results suggested the potential of these structures to boost the development of innovative tubular structures for tissue engineering approaches.

Tissue engineered constructs based on human adipose tissue derived stem cells and starch-based scaffolds for allogeneic approaches in tissue engineering and regenerative medicine

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The combination of natural-origin materials with mesenchymal stem cells (MSCs) from different sources have been suggested as suitable constructs for Tissue Engineering and Regenerative Medicine (TERM). Considering the importance of the host immune response to implanted tissue engineered constructs (TECs) and the immunomodulatory role assigned to MSCs and to adipose derived stem cells (ASCs) in particular, this work aimed at understanding the impact of SPCL-hASCs based TECs in the host inflammatory/immune system cells. The in vitro profile of inflammatory and anti-inflammatory cytokines expressed by macrophages (MØ) and dendritic cells (DCs), in contact with the scaffolds and the SPCL-hASCs based TECs, was evaluated by quantitative polymerase chain reaction (qRT-PCR). After 12 h and 24 h of direct contact, it was found that the level of expression of IL-10, IL-4, IL-6 and TNF both in MØ and DCs was bellow the detection limit. Additionally, the scaffolds and the SPCL-hASCs based TECs were intraperitoneally implanted in Balb/c mice in order to assess the expression of specific biomarkers of relevant immune cells through qRT-PCR, namely MØ, polymorphonuclear neutrophils (PMNS), B and T lymphocytes and DCs. Over the time course of the experiment comprising 7 days it was possible to verify that the SPCL scaffold seemed to trigger a less aggressive inflammatory response seen by a diminished expression of the evaluated immune cell markers when compared to the negative control. When comparing the TECs to the controls and the scaffold, TECs seemed to have the most promising results, that is, a diminished response throughout all the studied inflammatory/immune cell types was verified. These findings suggest that the combination of SPCL scaffolds with hASCs offer a promise strategy for allogeneic approaches in the TERM field, also corroborating the reduced immunogenic properties of hASCs.
**TS29**

**Antibacterial silica-borate glasses for bone tissue engineering applications**

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One of the major problems in bone tissue engineering/reconstruction is the possibility of occurring bacterial contamination during the surgical procedure. Bacterial strains such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* proved to be very difficult to eliminate from bone grafts [1]. Silica-based bioglasses, namely from the silica-borate system is gaining interest in this field [2]. Knowing in advance that boron has antibacterial properties [3]; it is relevant to evaluate the anti-bacterial properties of silica-borate glass formulations [4, 5]. In fact, bioglasses have been used in bone tissue engineering for several decades due to their capacity to improve, for example, the scaffolds mechanical performance, bioactivity or to promote osteoinduction. In this view, the substitution of the current bioglasses with one that, added to the previous listed properties, present an inherent antibacterial activity is a relevant approach in the development of bone tissue engineering strategies. In this context, we synthesized silica-borate glass compositions by melt quenching, where a suitable composition of starting chemicals (e.g. B₂O₃, CaCO₃, etc.) are mixed in a crucible and red to a temperature capable of melting the whole mixture (typical temperatures between 1000 °C and 1300 °C). The synthesized glass particles were ground to size that were tested for their cytotoxicity. Glass compositions of general formula 0.20B₂O₃:0.40SiO₂:0.35CaO:(0.35-x-y)SrO:0.05Na₂O (molar ratio, where x, y = 0.35 or 0.00, and x ≠ y) were synthesized and biologically tested. The cytotoxicity assessment was made by direct contact of each glass sample (9, 18 and 40 mg/mL) with human osteosarcoma cell line SaOs-2 at a density of 1.5E10⁴ cells/mL during 7 days of incubation (37 °C and 5% CO₂ atmosphere). For 1, 3 and 7 days of culture, the cell proliferation (DNA quantification) and metabolic activity (MTS) were monitored. The in vitro results (DNA and MTS) allow us to state that cells remain viable during the 7 days of culture. Preliminary agar diffusion assay tests, measuring the antimicrobial effect of the glass compositions against *S. aureus* and *P. aeruginosa* showed that the 0.20B₂O₃:0.40SiO₂:0.35SrO:0.05Na₂O glass inhibits bacterial growth at 9 and 18 mg/mL. Further studies are required to test different glass compositions (with varying proportions of SrO); glass concentrations in the medium; and bacterial strains.

**References:**


**TS30**

**Semiconductor gellan gum based composite hydrogels for tissue engineering applications**

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Semiconductor hydrogels can be developed by combining the intrinsic electrical properties of semiconductors with the specific characteristics of hydrogels. These hydrogels have recently attracted much attention for applications in tissue engineering, especially formulations incorporating pyrroly and excellent biocompatibility. Several studies have reported that electrical stimulation influences the migration, proliferation and differentiation of stem cells and other cell lines [1]. The goal of this work is to use in situ chemical polymerization of polypyrrole (PPy) with gellan gum (GG) in order to obtain a new generation of semiconductor composite hydrogels. For the synthesis of GG/PPy composites, GG at 1.25% (w/v) final concentration was prepared in distilled water at room temperature. The solution was then heated under stirring at 90°C for 20 min. Temperature was decreased to 65°C and Py was added under vigorous agitation. The crosslinker solution (CaCl₂, 0.18%) was added at 50°C. After 2 h, GG/Py composite hydrogels were obtained. In a further step, GG/Py samples were immersed in a solution of oxidizing agent in PBS and the reaction was carried out for 18 h under agitation at room temperature. Finally, the samples were frozen at ~80°C for 48 h and lyophilized. The characterization of GG, GG/PPy and PPy samples was performed by scanning electron microscopy (SEM). The incorporation of PPy in the gellan gel was confirmed by SEM analysis. The coating with PPy increases the thickness of each sheet in 3 fold when compared with GG samples. Conductivity tests were also performed. For cytotoxicity assay, the samples were rehydrated with complete culture medium. MTS and DNA quantification assays were performed to evaluate the metabolic activity and proliferation of L929 fibroblast cells after 1, 3 and 7 days in culture with GG, GG/PPy and PPy samples. MTS assays clearly indicate a proportional relation between the cell viability and the PPy concentration: higher concentrations of PPy resulted in lower cell viability. These results show that lower concentration of PPy incorporated in the GG hydrogels can provide an adequate electrical stimulus to improve cell behavior. In conclusion, semiconductor hydrogels can be an excellent platform for tissue engineering and electrochemical therapy applications.

**References:**

Combinatorial analysis of marine based biomaterials: high-throughput analysis of the effect of nanostructured multilayers on cell behaviour

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In a marine environment, specific proteins are secreted by mussels and used as a biogluue to stick to a surface allowing generate irreversible bonding. Adhesive secreted proteins of mussels present an unusual amino acid 3,4-dihydroxyphenylalanine (DOPA). Inspired by the structure and properties of mussel adhesive proteins, layer-by-layer (LbL) coatings based on polymers that contain catechol groups were developed. We used dopamine-modified hyaluronic acid (HA-DN) prepared by carbodiimide chemistry to form thin and surface-adherent dopamine films. The multilayer films were developed by electrostatic interactions using chitosan (CHT) as polycation and HA-DN as polyanion. Multilayers films of CHT and HA were used as control. The formation of these films was investigated in-situ by quartz crystal microbalance with dissipation monitoring (QCM-D). Afterwards, many combinations of the marine inspired biomaterials were analysed in a high-throughput (HTS) way. Such multilayers were constructed and individually disposed on isolated transparent spots, patterned onto biomimetic superhydrophobic substrates. The adhesion properties of the coatings in the chips were also analyzed. In vitro tests using two distinct cell sources were carried out to evaluate the biological performance of the different combinations of multilayers that could be useful in different biomedical applications, including tissue engineering.

The development of high-throughput and combinatorial technologies is helping to speed up research that is applicable in many areas of chemistry, engineering and biology. We propose a simple, versatile high-efficient and new superhydrophobic platform, which permits to arrange of quasi-spherical aqueous-based droplets with the capability to support and monitor a series of chemical/biological reactions on a lab-on-chip scale. Superhydrophobic biomimetic surface based on so-called lotus effect were produced onto which array of microindentations to fix liquid droplets, based on the rose petals effect. Such platforms sustain stable arrays of droplets with microliter volumes allowing to isolate and confine different combinations of biological materials. We demonstrate that it is possible to add agitation capability using magnet microspheres, enabling to create mechanical stress inside the microliter-size droplets. Different experiments were also performed to demonstrate the suitability of the developed platform, including: (i) the efficacy of the mechanical agitation in a simple physical process, namely by following the dissolution of salt crystals inside the droplets; (ii) the monitoring of a chemical reaction, namely the crosslinking of chitosan with genipin with different concentrations of reagents; (iii) the evaluation of cell viability under different pHs; (iv) the evaluation of the cytotoxicity of drugs in cells spheroids, developed by gravity in the suspended droplets. Such technology has potential to be used in many biomedical applications, such as drug screening and biomaterials development.
TS33
Indirect co-cultures of stem cells with chondrocytes for cartilage tissue engineering using PCL electrospun nanofiber meshes
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Mesenchymal Stem Cells (MSCs) have been recognized for their ability to differentiate into cells of different tissues such as bone, cartilage or adipose tissue, and therefore might be of interest for potential therapeutic strategies. These cells are induced to differentiate by growth factors supplementation in culture medium that will trigger differentiation in the desired cell type. Chondrocytes are responsible for maintaining the extracellular matrix (ECM) integrity of articular cartilage. Chondrocytes have been shown to release growth factors that can ultimately induce chondrogenic differentiation of undifferentiated cells, for example MSCs. It is well known that chondrocytes tend to de-differentiate when in 2D culture, losing their ability to produce a rich ECM. In this process occurs a shift from collagen type II production to collagen type I, among other factors, giving rise to a fibrocartilage tissue. In order to overcome this problem, several tissue engineering strategies have been proposed, involving different combinations of cells, including the use of co-cultures. The present work presents a co-culture strategy using human articular chondrocytes and stem cells (Wharton’s jelly stem cells) for cartilage-like tissue production. We aimed at assessing the paracrine effect that chondrocytes may have on stem cells by co-culturing directly both cells on the two faces of NFMs. The aim is to allow communication of the two cells communities by soluble factors released, but not having direct contact between them. Polycaprolactone (PCL) nanofiber meshes (NFM) were produced by electrospinning. The NFMs were further placed into inserts (two in each insert) in order to allow seeding each type of cells in opposite faces of the NFMs. Cells were isolated from human samples collected at the local hospital, under donors’ informed consent. After cells expansion, chondrocytes were seeded on the top of the NFMs, whereas stem cells were seeded on the bottom of the NFMs. Controls were performed by seeding chondrocytes or stem cells in NFM. For evaluation of cell viability, proliferation and distribution within the scaffolds, DNA, Alamar Blue and SEM methods were used. Chondrogenic differentiation was evaluated using histological staining, glycosaminoglycan quantification, qRT-PCR and immunocytochemical. Cells kept viable along the experiment. Stem cells were able to over express cartilage related genes such as aggrecan, sox9 and collagen type II when compared to the undifferentiated controls. Articular chondrocytes induced the chondrogenic differentiation of stem cells and ECM formation. The obtained results showed that this new strategy enables the development of new therapies for cartilage repair.

TS34
Magnetic-responsive hydrogels for cartilage tissue engineering
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The use of magnetic nanoparticles (MNPs) has been explored as an alternative approach to overcome current limitations of regenerative medicine strategies. Cell engineering approaches where MNPs are incorporated within three-dimensional constructs, such as scaffolds or hydrogels may constitute a novel and attractive approach towards the development of a magnetically-responsive system. These systems would enable remote controlled actions over tissue engineered constructs in vitro and in vivo. Moreover, growing evidence suggests that the application of a magnetic field may enhance biological performance over commonly used static culture conditions providing stimulation for cell proliferation, migration and differentiation. In this work we analyze the role of magnetic stimulation on the behavior of human adipose derived stem cells (hASCs) laden in k-carrageenan hydrogels aiming at cartilage tissue engineering approaches. Thermo-responsive natural-based α-k-carrageenan hydrogels were used as 3D templates since previous studies report the adequate environment provided by these materials to support the viability and chondrogenic differentiation of several types of cells. α-k-carrageenan (α-k) was mixed with MNPs in different ratios, namely 2.5, 5 and 10%. Human ASCs previously isolated from surplus tissues from elective plastic surgery procedures, were encapsulated in these k-carr-MNP hydrogels and cultured in vitro for up to 21 days in chondrogenic culture medium either in the presence or absence of magnetic stimulation generated by a bioreactor device. The hASCs-laden constructs were assessed for cell viability, cell proliferation as well as deposition of a cartilaginous-like extracellular matrix. Human ASCs appear to preferentially adhere to MNPs as they could be found in higher concentrations in regions enriched with the magnetic component. The presence of MNPs within the α-k-carrageenan hydrogels did not significantly influence the viability or proliferation of encapsulated hASCs, whose values were similar to hydrogel MNP-free controls. Results also indicate that the formation of vacuoles typically observed in chondrocytic cells, was noticed in cells laden k-carr-MNP hydrogels supplemented with chondrogenic medium. Stem cell performance on k-carr-MNP hydrogels can be modulated by the presence of MNPs stimulated by a magnetic field. Magnetic responsive hydrogels can stimulate hASCs towards chondrogenic differentiation, without affecting cell viability or cell proliferation rates. Therefore, magnetic-based systems may provide new opportunities in regenerative medicine applications towards cartilage engineered tissues.

Reference:
Nano and micro fiber combined scaffolds have been produced in order to mimic the biophysical structure of natural extracellular matrix (ECM). Herein the strategy was to combine layers of polycaprolactone (PCL) electrospun nanofiber meshes (NFM) every two consecutive layers of starch-polycaprolactone rapid prototyped microfibers (SPCL-RP). Human umbilical cord Wharton's Jelly stem cells (hWJSCs) were isolated, seeded and cultured for 21 days on hierarchical starch based scaffolds, under osteogenic differentiation conditions, to evaluate the influence of the integrated nanofiber meshes on cell entrainment and on osteogenic differentiation. In vitro biological data confirmed that hierarchical starch-based fibrous scaffolds showed enhanced cell entrainment when compared to S. Claudio do Barco, 4800-058 Industrial da Gandra, S. Claudio do Barco, 4800-909 Taipas, Guimarães, Portugal; 3ICVS/3B’s Associate Laboratory, Braga/Guimarães, Portugal; 4Life and Health Sciences Research Institute ICVS, School of Health Sciences, University of Minho, Braga, Portugal; 5Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal; 6Department of Mathematics and Applications, Mathematical Research Centre CMAT, University of Minho, Campus de Azurém, 4800-058 Guimarães, Portugal.

The development of strategies based on the use of scaffolds for Tissue Engineering and Regenerative Medicine (TERM) has been hindered by the inability of researchers to present solutions to overcome problems mainly related with the biocompatibility of materials and in vivo perfusion of the 3D structures. It is in this context that scaffold-free methodologies are being presented as increasingly attractive strategies for TERM. Scaffold-free approaches in general rely on the production of extracellular matrix (ECM) by the cells of interest. However, the creation of an ECM robust enough for use in TERM is many times a challenge. Therefore, there is an important need to develop protocols that boost cell’s ability to produce ECM. One way to achieve this is to expose cells to an environment crowded with adequate macromolecules in order to mimic the physiological cellular milieu. Dextran sulphate (DS) and Ficoll (Fc) have been suggested as compounds capable of increasing ECM deposition. The involved mechanism is closely related to the increase in enzyme-mediated collagen deposition. In the present work, we hypothesized that the use of DS or of a combination of Fc of different molecular weights (Fc/70/Fc/400) as crowders in culture medium could increase the robustness of the ECM produced by human fibroblasts (hFb) or human adipose-derived Stem Cells (hASCs). 5x10^6 hFB or hASC were seeded on wells of 24 and 48 well plates, and cultured for 24 h in α-MEM supplemented with10% FBS and 1% antibiotics. After the first 24 h, the medium was replaced by fresh medium supplemented with 1% FBS and a) 50 μg/mL of Ascorbic Acid, b) 50 μg/mL of Dextran Sulphate, or c) 37.5 mg/mL of Fc/70 + 25 mg/mL of Fc/400. Cells were cultured for further 2 and 5 days. dsDNA quantification showed that in both conditions b) and c), and independently of the cell type, cell proliferation was significantly reduced. ECM production was evaluated by quantifying the deposited collagen using a semi-quantitative Sirus Red kit (Picrosirius, Chondrex, USA). Collagen quantification, normalized with dsDNA, demonstrated that for both cells types, the presence of either DS or Fc/70/Fc/400 resulted in the decrease of ECM deposition. In conclusion, the use of DS and Fc/70/Fc under the conditions herein described failed to increase the ECM production by both hFb and hASCs.

Acknowledgements: This research has been funded by the EU Seventh Framework Programme under grant agreement FP7-KBBE-2010-4-266033-SPECIAL and by the Portuguese Foundation for Science and Technology (FCT)-funded project Skinengineering (PTDC/SAU-OSM/099422/2008).

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1. Chen et al. BJP 2009

Abstracts List
Biomedical exploitation of squid chitosan using particle aggregation derived composite scaffolds

LC Reys, SS Silva, RP Pirracio, MB Bontinguiça, R Comesa, MJ dos Reis, RL Reis, LPS Silva, JL Reys, LS Silva, JF Mano, TH Silva, and RL Reis

In the last decades, marine organisms have been the focus of considerable attention as potential source of valuable materials. Some important examples are β-chitin isolated from the endoskeleton of squids and hydroxyapatite (HA) produced from fish-bones. β-chitin is a structural polysaccharide more reactive than the most common α crystallographic form, thus allowing the production of chitosan with high deacetylation degree without a significant effect on molecular weight. HA (Ca10(PO4)6(H2O)2), found in fish bones, has special importance in biomedical field due to its similarities with the mineral constituents of human bones. In this work, the biomedical potential of squid chitosan and fish hydroxyapatite was assessed by processing them into composite porous structures by particle agglomeration for tissue engineering scaffolding. For that, β-chitin was isolated from endoskeleton of giant squid Dosidicus gigas and further deacetylated to produce chitosan. Hydroxyapatite nanoparticles (nHA) were synthesized from fish bones by pulsed laser in deionized water. Subsequently, a solution of 2% chitosan and 3% nHA in 1% acetic acid was extruded through a syringe and further randomly packed into a mould to render porous structures by particle aggregation promoted by physical or thermal interaction. The developed structures are characterized by low porosity but high interconnectivity, being essentially semi-crystalline, with a compression modulus of 48 MPa. To examine cell behavior in the developed structures, 1x10^5 human adipose derived stem cells (hASC) were seeded in the nanocomposite scaffolds and in chitosan-alone scaffolds. Preliminary results after 7 days of culture have shown that the nHA scaffolds were more favorable for hASC proliferation in comparison with chitosan scaffolds, as reflected in the increase of 30% in the dsDNA quantity. These findings indicated that the chitosan/nHA structures can be a good candidate for biomedical applications, namely on bone regeneration.

A cell spanning IKVAV expressing peptide for treatment of spinal cord injury

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In this study, the biomedical potential of squid chitosan and fish hydroxyapatite was assessed by processing them into composite porous structures by particle agglomeration for tissue engineering scaffolding. For that, β-chitin was isolated from endoskeleton of giant squid Dosidicus gigas and further deacetylated to produce chitosan. Hydroxyapatite nanoparticles (nHA) were synthesized from fish bones by pulsed laser in deionized water. Subsequently, a solution of 2% chitosan and 3% nHA in 1% acetic acid was extruded through a syringe and further randomly packed into a mould to render porous structures by particle aggregation promoted by physical or thermal interaction. The developed structures are characterized by low porosity but high interconnectivity, being essentially semi-crystalline, with a compression modulus of 48 MPa. To examine cell behavior in the developed structures, 1x10^5 human adipose derived stem cells (hASC) were seeded in the nanocomposite scaffolds and in chitosan-alone scaffolds. Preliminary results after 7 days of culture have shown that the nHA scaffolds were more favorable for hASC proliferation in comparison with chitosan scaffolds, as reflected in the increase of 30% in the dsDNA quantity. These findings indicated that the chitosan/nHA structures can be a good candidate for biomedical applications, namely on bone regeneration.

Spinal cord regeneration following local treatment with a membrane spanning peptide (MSP) expressing the IKVAV epitope was assessed following compression injury in Balb-c mice. The day after hemilaminectomy and compression injury, mice were treated with one of the following: isoleucine-lysine-valine-alanine-valine (IKVAV), IKVAV-MSP, peptide and mannitol/saline (vehicle). Functional improvement in movement was assessed daily using Basso Mouse Scale (BMS) and spinal cord segments were studied histologically 28 days after injury. The BMS score for the IKVAV-MSP group increased significantly (P < 0.05) compared to IKVAV-control, and MSP-control groups beginning on day 13. The number of protoplasmic astrocytes in the IKVAV-MSP mice was significantly increased compared to IKVAV, mannitol and normal groups but not with the MSP-control group (P < 0.001). Neuron and muscle bundle size were also increased significantly (P < 0.05 and P < 0.007, resp.) in the IKVAV-MSP group compared to other treatment groups. The observations in this study demonstrated that it is possible to promote functional recovery after SCI using bioactive IKVAV presenting cell membrane spanning peptides.
Characterization of free-standing multilayer membranes made of chitosan and alginate

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Objective: Free-standing films have increasing applications in the biomedical field as drug delivery systems, for wound healing and tissue regeneration. In this work, we produced free-standing multilayer membrane made of chitosan (CHI) and alginate (ALG), by detaching a polyelectrolyte multilayer film from its underlying substrate without any postprocessing step. The morphology and the chemical properties of the CHI/ALG multilayer films were characterized.

Methods: In this work, the buildup of free-standing multilayer films made of CHI and ALG was investigated. Several conditions were tested to follow the film growth in order to get thick films. The CHI/ALG free-standing films were characterized by Fourier transform infrared spectroscopy (FTIR), atomic force microscopy (AFM) and by scanning electron microscopy (SEM). Permeability tests were performed using FITC-dextran, with several molecular weights, as a drug model molecule.

Results and Discussion: The produced membranes can be detached from an underlying inert substrate without any postprocessing step. Permeability experiments on these membranes revealed that the permeation of FITC-dextran depended greatly on its molecular weight.

Conclusions: The production of free-standing films permits the direct experimental determination of many physical properties of fundamental significance such as ion permeation and mechanical properties that can be tuned for real-world applications. These free-standing films are easy detachable, easy to handle, stable in the presence of physiological solutions and biocompatible, demonstrating potential for applications in tissue engineering and regenerative medicine.

Immobilization of bioactive factor-loaded liposomes at the surface of electrospun nanofibers targeting tissue engineering strategies

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The ability to manipulate and control the surface properties is of crucial importance in the designing of scaffolds for Tissue Engineering (TE) and Regenerative Medicine. Electrospun nanofibers (NFMs), due to their morphology and fibrous structure have received much attention as potential biomedical devices, TE scaffolds and drug delivery carriers. Liposomes, a nanoparticle release system made by physiological material (phospholipids), hold tremendous promise as release systems. Liposomes may be combined with scaffolds to maintain a sustained and local delivery of the loaded drugs. The main objective of the present study is to evaluate the efficacy of dexamethasone (Dex) loaded liposomes immobilized on the surface of polycaprolactone (PCL) electrospun nanofiber meshes (NFMs) as release system, for the induction of the osteogenic differentiation of human bone marrow-derived mesenchymal stem cells (hBMSCs). The PCL NFMs surfaces were activated using the UV-Ozone irradiation technique. Aminolysis was performed to insert amine groups onto the NFMs surface. Afterwards, SH groups were inserted at the surface of the NFMs through the reaction of the aminated surfaces with 2-iminothiolane. Ellman’s reagent method was used to quantify the SH groups onto the NFMs surfaces. Dex-loaded liposomes were covalently immobilized at the surface of chemically functionalized electrospun PCL NFMs. The in vitro release profile demonstrated a sustained release of Dex during 21 days, after an initial burst release of 12 h. Biological assays showed that Dex-loaded liposomes immobilized at the surface of electrospun PCL NFMs did not exhibit any cytotoxic effect, promoting the osteogenic differentiation of hBMSCs. We herein validate the concept of using liposomes immobilized at the surface of a nanostructured fibrous system to be used as an advanced cell carrier device with autonomous release of growth/differentiation factors relevant for tissue engineering and regenerative medicine strategies.
Superhydrophobic surfaces (SHS) are characterized for exhibit extreme water repellency. Where water droplets roll easily and have a contact angle higher than 150°. The inspiration to produce artificial SHS comes from nature, the Lotus leaf. Hierarchical surface topographies at micro/nanoscale are critical for this effect. On biomedical and tissue engineering fields several applications for SHS has been developed. Such as microfluidic platforms to perform studies in mimic in vivo environment similar to human body. SHS are also used to produced spherical particles without using any precipitation bath. The method permit to produce particles for controlled drug delivery in one step with high encapsulation efficiency. Cells was also encapsulated on a system to be used in tissue regeneration was obtained. Other promising application for SHS is high-throughput screening. Using SHS, platforms to analyze several materials/formulations at the same time were developed. These platforms permit to perform combinatorial studies with cells/biomaterial to screen cytoocompatibility. Several strategies were developed to produce SHS: polymer reconformation, template method or sol–gel processing. One strategy involves producing roughness by silica micro/nanoparticles deposition on glass slides. Others is to use natural structures as templates that exhibit the necessary hierarchical structure. These two strategies inspired us to develop a new approach to generate SHS. We use silica-based structures already available in nature to create the necessary hierarchical topography. We use diatomaceous earth directly on the surface and not as templates. The diatomaceous exoskeletons are microstructures with nanotextures. These micromstructures were used to coat smooth surfaces and create a hierarchical roughness on surfaces. By fluorosilanization a SHS was achieved. The wettability of the produced surfaces can be precisely controlled by exposing the substrates to plasma treatment for specific times. The control in space of the treatment can be used to imprint hydrophilic patterns on the SHS. This make promising the use of developed SHS in controlling the treatment can be used to imprint hydrophilic patterns. The versatility of the developed strategy can be applied in different kinds of substrates. The versatility of the developed method to produce SHS show high potential for biomedical and tissue engineering applications.

References:
TS43
High-throughput skeletal stem cell separation using magnetic labelling and microfluidic sorting
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Medical advances have led to a welcome increase in life expectancy. However, this progress presents its own new challenges: increases in age-related diseases, and associated reductions in quality of life, have substantial socio-economic cost. OA is the most common form of arthritis worldwide and the sixth leading cause of disability and in severe cases, necessitating joint replacement using non-biological prostheses. A major limitation in the use of prostheses is the risk of infection, dislocation, mechanical mismatch and functional failure; all leading to revised implants and further surgery. Cell-based therapies are currently some of the most exciting and promising areas for bone disease treatment and reparative medicine. SSCs present in bone marrow (BM) contribute to the regeneration of mesenchymal tissues such as bone, muscle, ligament, tendon and stroma. However, despite intensive research interest, there are currently no reliable methods to isolate (or enrich sufficiently) homogeneous skeletal stem cell populations needed for these strategies given their paucity, less than 0.01%, in bone marrow. This research seeks to develop SSC isolation techniques using unique microfluidic strategies. Traditional immunological sorting methods such as fluorescence/magnetic activated cell sorting (FACS/MACS) can be used to isolate SSCs according to surface marker expression. Both techniques have limitations with regards to purity (~70%), cell viability (20-25% post sorting), running cost, mechanical complexity and the need for trained dedicated technicians, especially FACS. A microfluidic-based approach offers reduced running costs and enhanced homogeneous stem cell and progenitor enrichment. Here, we detail an innovative approach to isolate, sort and characterise SSCs from human BM stromal cells (HBMSCs) using a microfluidic device. Functionalised super-paramagnetic beads with adsorbed STR-1 antibody were used to target the SSC population. Immunomagnetically labelled cells experience a drag force due to a magnetic field generated by thirty permanent neodymium magnets. The system, designed on the same principles as conventional MACS has the added advantage of continuous flow enabling labelled cell separation. Experiments have been performed using polystyrene beads labelled with magnetic nanoparticles to simulate the target cells behaviour, while current work is focussed on isolation of STR-1+ cells from heterogeneous MG63 human osteosarcoma cells. The final phase will be the assessment of STR-1+ cell separation from whole adult HBMSC populations using this system. This microfluidic approach offers an innovative approach to skeletal stem cell enrichment with significant therapeutic potential therein for cell isolation for skeletal evaluation and application.

TS44
Polysaccharide-based nanostructured multilayers with distinct sulfated and aminated composition to improve cells response and biomineralization
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In vitro cell expansion, differentiation for further cell transplantation and biomaterials-cell fundamental assays are still performed more often on inert 2D surfaces than on 3D culture. 3D systems do not allow an easy cell monitoring and may demand higher cell density, being more costly and time consuming. Inert surfaces (polystyrene, biodegradable thermoplastics or metals) neither resemble the extracellular matrix (ECM) milieu nor trigger intercellular signaling. Several studies are focused on surface modification and on the correlation of the surface properties such as roughness, wettability and chemistry with cell behavior with regard to the effects on cell adhesion, morphology, proliferation, survival and differentiation. Proteins, hormones, small peptides, cytokines, inorganic molecules, sulfated and non-polysaccharides (PS) compose the natural 3D ECM milieu. PS vary on the sulfur content, sulfonic group (Sg) position and on base units. Sulfonated and sulfated PS have intrinsic very high affinity towards growth factors and positively charged molecules through the functional groups turning the combination of them very bioactive hybrids mats. Current surface modification models make the transposition to 3D systems complicate. Layer-by-Layer (LbL) assembling is a versatile technique to coat any 2D/3D structure with polyelectrolytes (PE) which coatings properties can be modelled and controlled. Herein, LbL was employed to develop 2D models to verify the ability of sulfated and aminated coatings to improve cell function using PS form marine origin: chitosan (Chi) and carrageenans (Cars). Chi and Cars have equivalent functional groups to the ones that are naturally found in the ECM: -NH2, -OSO3H, and -OH. CHI and Cars - PT Government Associate Laboratory, Braga/Guimarães, Portugal

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Bone tissue engineering (BTE) holds the potential to develop functional substrates for damaged tissue using a scaffold-cell hybrid. The optimization of the surface roughness of orthopedic implants that could stimulate osteogenic differentiation of mesenchymal stem cells (MSCs) would have significant therapeutic potential. Yet there is still a lack of systematic studies on the surface properties of FDA-approved biodegradable polymers, such as polycaprolactone, relevant for BTE and Regenerative Medicine and its effect over uncommitted cells such as human bone marrow (hBM)-MSCs. We produced a surface gradient on PCL films varying from the submicron to the micrometer range of average roughness \( R_a \) \( < 0.23 \) \( \mu m \) to \( > 4.71 \). The gradients were produced by hot-embossing of a polyvinylsiloxane master cast from an aluminum substrate that was previously sand-blasted and chemically polished at a controlled rate. Human bone marrow MSCs (hBM-MSCs) were cultured for 21 days in static conditions on the PCL roughness gradients and on tissue culture polystyrene (TCP5), which was used as a reference material. The osteogenic commitment of the cell population was investigated by quantification of immuno-detection of alkaline phosphatase (ALP) and collagen type 1 alpha 1 (COL1A1) markers, and also by quantification of calcium mineral deposition stained by alizarin red. To evaluate the surface morphological cues, such as rounded or cuboidal shapes, we concomitantly stained the hBM-MSCs for F-actin that constitutes the cytoskeleton backbone. We showed that roughness values between 2.07 and 3.13 \( \pm 0.23 \) \( \mu m \) significantly accelerated the commitment of hBM-MSCs, translated by ALP activity at 4 days of culture, in comparison to 7 and 10 days at the submicron \( R_a \) range and TCP5 respectively. This \( R_a \) window strongly supported COL1A1 deposition and significantly higher matrix calcification than the surface roughness at the submicron range and TCP5 (0.001 < \( P < 0.05 \)). Moreover, our results are consistent with a change of the cell population morphology, from an elongated to a cuboidal shape, with increasing \( R_a \) of the PCL gradient. The cuboidal shape registered at the micrometer side of the roughness gradient has already been reported as characteristic of mature osteoblasts. Our results highlighted the potential of specific roughness windows of PCL to modulate hBM-MSCs through optimized roughness, with implications for bone regeneration strategies and for medical device manufacturing.

Skin Tissue Engineering (TE) represents the most effective way to target skin regeneration, namely in the case of massive tissue loss and burns, where an effective intervention is crucial to minimize wound contraction and scar formation. However, a frequent and fundamental problem resides on the insufficient re-vascularization after grafting, that compromises the strategy employed and that has been described to significantly contribute to fibrosis. Pre-vascularization of skin substitutes, despite the limitation still encountered with the source of endothelial cells, turned out to be a promising approach for a more efficient inoculation, consequently improving wound healing. To meet this, we proposed a skin TE strategy that combines an off-the-shelf scaffold with human adipose stem cells (hASCs) and adipose tissue microvascular endothelial cells (hAECs) to promote skin tissue regeneration by modulating neovascularization and the intricate cascade of events that drive skin wound healing. The innovative character of the proposed approach relies on taking advantage of a powerful cell-machinery obtained from a single cell source combined with a gellan gum-hyaluronic acid spongy-like hydrogel (GG-HA), which, unlike traditional hydrogels, hold cell adhesive properties and attractive mechanical performance. Stable and off-the-shelf dried GG-HA polymeric networks, rapidly re-hydrated at the time of cell seeding, then depicting features of both sponges and hydrogels, enabled the natural cell entrapment/encapsulation and consequent attachment and interaction. After transplantation to full-thickness excisional mouse wounds, GG-HA spongy-like hydrogels constructs showed to facilitate the early inflammatory cell infiltration, translated by a dense granulation tissue formation, resulting in a rapid degradation and matrix remodeling, and complete wound closure and reepithelization. More importantly, GG-HA spongy-like hydrogels constructs promoted neovascularization, further enhanced by the presence of hAECs that directly incorporated the neovessels formed. By uniting an off-the-shelf dried network and two cell types obtained in a relatively short timeframe from the same source, we were able to demonstrate the possibility of creating a clinically relevant skin tissue substitute that acts in promoting neoskin vascularization.
**TS47**

Organic-inorganic nanostructured multilayers for calcium phosphate biomineralization

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The weak fixation of biomaterials within the bone structure is one of the major reasons of implants failures. Calcium phosphate (CaP) coatings are used in bone tissue engineering to improve implant osseointegration by enhancing cellular adhesion, proliferation and differentiation, leading to a tight and stable junction between implant and host bone. It has also been observed that materials compatible with bone tissue either have a CaP coating or develop such a calcified surface upon implantation. Thus, the development of bioactive coatings becomes essential for further improvement of integration with the surrounding tissue. However, most of current applied CaP coatings methods (e.g. physical vapor deposition), cannot be applied to complex shapes and porous implants, provide poor structural control over the coating and prevent incorporation of bioactive organic compounds (e.g. antibiotics, growth factors) because of the used harsh processing conditions. Layer-by-layer (LbL) is a versatile technology that permits the building-up of multilayered polyelectrolyte films in mild conditions based on the alternate adsorption of cationic and anionic elements that can integrate bioactive compounds. As it is recognized in nature’s biomineralization process the presence of an organic template to induce mineral deposition, this work investigate a ion based biomimetic method where all the process is based on LbL methodology made of weak natural-origin polyelectrolytes. A nanostructured multilayer component, with 5 or 10 bilayers, was produced initially using chitosan and chondroitin sulphate polyelectrolyte biopolymers, which possess similarities with the extracellular matrix and good biocompatibility. The multilayers are then rinsed with a sequential passing of solutions containing Ca²⁺ and PO₄³⁻ ions. The formation of CaP over the polyelectrolyte multilayers was confirmed by QCM-D, SEM and EDX. The outcomes show that 10 polyelectrolyte bilayer condition behaved as a better site for initiating the formation of CaP as the precipitation occur at earlier stages than in 5 polyelectrolyte bilayers one. This denotes that higher number of bilayers could hold the CaP crystals more efficiently. This work achieved uniform coatings that can be applied to any surface with access to the liquid media in a low-temperature method, which potentiates the manufacture of effective bioactive biomaterials with great potential in orthopedic applications.

**TS48**

Mouse fetal neural stem cell preparation and brain transplantation using alginate hydrogels

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The promise of stem cell therapy to achieve tissue regeneration and replace the lost cells is in particular difficult to achieve in the brain. The three dimensional structure of the complicated network of neural cell, where new cells are rarely added in the mammalian brain after birth, represent a major challenge for the incorporation of the transplanted cells. To enhance the abilities of neural stem cells to be incorporated in the brain in particular, the neural stem cells were cultivated using alginate hydrogels. The cells were isolated from the brains of mouse fetuses 14 days old, and cultivated within the alginate beds of standardized 3 mm diameter. Their survival, differentiation and the behavior after the transplantation were followed in different alginate concentrations. The neural stem cells survived and differentiated within alginate beds. The neural markers were present after differentiation. In particular, the alginate concentration suitable for brain transplantation was investigated. The results showed that alginate hydrogels have potential to enhance the abilities of neural stem cells and to serve as a biomaterial supporting their transplantation to the brain. The study was supported by EU FP7 grant GlowBrain (REGPOT–2012–CT2012–316120).
Bioinspired superhydrophobic patterned surfaces as chips for high-throughput analysis of biomaterials viscoelastic properties

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Tissue-constituent cells are usually anchorage-dependent cells. Their viability is compromised when they are in a fluid suspension. The adhesion of these cells in the body occurs in solid elastic tissues. Mechanical properties of biomaterials were reported to have a role in modulating cells response, affecting their function and structure, as well as the direction of differentiation [1]. Cells like neurons, muscle cells, mesenchymal stem cells are examples of cells previously reported to be dependent on substrates stiffness. It is well known that interactions occurring in the area of tissue engineering and regeneration are not easily predictable. As such, we previously developed arrays of wettable spots in polystyrene superhydrophobic surfaces in order to use these structures as high-throughput platforms for biomaterials development. We patterned biomaterials precursors in the wettable spots, keeping them confined in such regions by the wettability contrast caused by the superhydrophobic surroundings. The method was used to study several cell-biomaterials interactions in 3D milieu. We studied the effect of distinct combinations of biomaterials in encapsulated cells [2], as well as cells-porous scaffolds interactions [3]. Adapted chips based on the same concept were also used to develop an on-chip drug-release quantification device [4]. By using the same type of chips developed for cells-biomaterials interactions studies, we aimed to adapt the system for the rapid study of miniaturized biomaterials viscoelastic properties. Most living tissues show a viscoelastic behavior, i.e., besides showing a particular stiffness, they have the ability to dissipate energy during cyclic stimulation. We used superhydrophobic chips to pattern miniaturized hydrogels, and adapted a mechanical dynamic analyzer (DMA) so on-chip viscoelastic properties of those materials could be assessed under physiological-like conditions. For the proof-of-concept we performed a three-factor combinatorial study targeting bone tissue engineering applications. A system consisting of distinct combinations of polymeric matrix concentration, crosslinking was systematically studied. We believe this system will facilitate the on-chip rapid study of miniaturized biomaterials for the future discovery of the role of viscoelastic properties of tissues and materials in cell response.

References:

High-throughput drug screening using cell spheroids in superhydrophobic patterned chips

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High-throughput studies in biotechnology areas such as drug screening and tissue engineering have been carried out mainly in 2D environment. However, low clinical and biological relevance of 2D models is well known. Both in tissues and tumor masses, cells lie in a 3D configuration organized in the self-secreted microenvironment: the extracellular matrix (ECM). In this milieu, cells interact in a totally natural manner, without the interaction of foreign factors, such as materials. The demand for studies using organotypic models is increasing, in order to improve the relevance of the findings achieved in these areas of study. A solution to create such organotypic models is the in vitro construction of cell spheroids. These are micro-masses of cells formed from a cell suspension, where the attachment of cells to any surface is not promoted. As such, cells tend to attach between themselves and form an organized mass, constituted by cells in combination with ECM. Some of spheroids are grown in order to mimic tumor models: the living spheroid structure contains a necrotic core, similarly to the native tumors. Cell spheroids are also useful as models for the development of complex microtissues [1], and can also be used as building blocks of larger tissues. Several methods to produce cell spheroids are reported in the literature. The hanging drop method is one of those methods: the cells are pulled to the concave bottom of a hanging drop by gravity effect, and tend to start the natural organization by cell-cell attachment and production of ECM. We propose the use of superhydrophobic surfaces patterned with wettable regions as platforms for the affordable and scale-up production of cell spheroids by the hanging drop method. Moreover, we used the platform - whose wettable regions are transparent and whose drop has its surface totally exposed to the external media allowing its facilitated manipulation – as high-throughput screening platform for drug testing and on-chip cell response analysis by microscopy. For the proof-of-concept we dispensed cell suspensions of distinct cell types (1929 and SaOs-2) with different cell concentrations in the array of wettable regions of the chips. We tested the effect of a cytostatic drug used in clinical practice (doxorubicin), also dispensed in a combinatorial logic in each spot of the chip. By on-chip microscopy analysis we proved the suitability of such platforms for drug screening using tumor-like models.

Reference:
TS51 Functional cell microcarriers- a new platform for cell separation and expansion
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The success of many stem cell applications in the biomedical field is highly dependent on the development of separation techniques for isolation and purification of cells with a very high yield and purity. Despite all the achievements made in the field over the past several years, new systems for effective cell separation are still needed. Previous work from our group demonstrated that functional chitosan films grafted with antibodies promote selective cell adhesion.1 Herein we developed chitosan microparticles able to capture a specific cell types based in the concept of antibody coating for cell sorting. Our goal was to create new biomaterial surfaces capable of recruit a specific cell population within a mixture, reducing cell manipulation and time-consuming allowing at the same time cell expansion. Such system acts as a microcarrier for cell expansion of a specific cell target. Microcarrier culture system offers the advantage of providing a larger surface area for the growth of anchor-age-dependent cells in a suspension culture system. Chitosan was chosen due to the excellent biocompatibility, gel forming properties, chemistry surface and low cell adhesion. This allows the modification with specific biochemical cues, for a controllable cell attachment. Here we develop functional biotinylated microparticles, such system allows tailoring microparticles to a variety of functional biomolecules. Here we tested the immobilization of antibodies to target specific cell types, CD31 for endothelial cells and CD90 for adipose stem cells. Primarily designed for an application in tissue engineering, two main challenges are accomplished with the herein presented microparticles: separation and scale-up expansion of specific cell type. The herein developed polymeric microparticles can also be used for directly deliver cells in vivo to repair and regenerate tissues.

References:

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TS52 Platelets lysate-based membranes for periodontal ligament regeneration
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The periodontal ligament (PDL) is a group of specialized connective tissue fibers that attach a tooth to the alveolar bone where it is deployed. These fibers help the tooth withstand the substantial compressive forces which occur during chewing and remain embedded in the bone. Periodontitis is a prevalent infection disease that causes the destruction of the tooth supportive tissues including the PDL. Given its low ability of regeneration in adult patients, concerted efforts have been made to accelerate periodontal tissue regeneration. Nevertheless, a strategy for predictable reconstruction of normal structure and functionality of periodontal damaged tissue is yet to be achieved. In this work, we present a novel membrane based on platelets lysate (PL) aiming for PDL regeneration. PL is a source of multiple growth factors (GFs) such as PDGF-BB, VEGF, and TGF-β1, which are prompt to induce wound healing and the recruitment of cells for tissue regeneration. In this work, we propose the development of PL-based membranes prepared by crosslinking PL proteins with genipin for periodontal tissue regeneration. Increasing concentrations of genipin (0.10, 0.18 and 0.25% w/v) were used to crosslink PL proteins to produce PL-based membranes. The resulting membranes showed increasing crosslinking density proportional to the crosslinker concentration. In addition, the morphological and mechanical features have shown to be dependent on the crosslinking degree of the PL membranes. The release of specific GFs was quantified by ELISA. Results show that the produced membranes are able to release the GFs contained in PL in a controlled manner and proportional to the crosslinking density, with a higher cumulative release for the samples with lower crosslinking density. In vitro assays were performed both using human adipose derived stem cells (hASCs) and periodontal ligament fibroblasts (hPDLFs). While no significant proliferation was detected when using hASCs, the hPDLFs showed good adhesion and proliferation on the membranes, suggesting its compatibility with PDL regeneration approaches. The PL-based membranes developed in this work, present high stiffness and elasticity and, consequently, a great potential in the regeneration of elastic and mechanically active tissues. Moreover these membranes have demonstrated to act as a valuable substrate for hPDLFs attachment and growth in 2D conditions and provide an environment rich in GFs with a major role in wound healing. These results suggest that it is possible to produce stable PL-based membranes crosslinked with genipin and that these membranes have great potential for future applications in the regeneration of PDL.
Potential of marine sponge collagen coatings for skin regeneration strategies

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Collagen is the most abundant protein in mammals and has found a wide range of applications in human health-related fields, namely cosmetics, pharmaceutical, dental, skin regeneration, ophthalmology, cardiac surgery and orthopaedics. Bovine and porcine bones and skins are the main industrial sources of collagen. However, due to religious constraints on the use of porcine products and to the risk of bovine spongiform encephalopathy (BSE) and other diseases posing to humans when infected bovine derived products are used, other sources of collagen are being pursued. Marine raw-materials have arisen on the last years and, in particular, marine sponges can be an important source of collagen. In this work, collagen was extracted from the marine sponge Chondrosia reniformis using different extraction methods: (i) 0.5 M acetic acid with 10% pepsin; (ii) 50 mM Tris-HCl with 1M NaCl; and (iii) 100 mM Tris-HCl, 10 mM EDTA, 8 M urea and 100 mM 2-mercaptoethanol. Cytotoxicity of the extracted collagen was assessed with L929 cell culture onto collagen coatings (5.5 µg/mL), with evaluation of metabolic activity (MTS assay) and cell proliferation (DNA quantification). Coatings of collagen extracted from C. reniformis are not cytotoxic and promoted proliferation. Moreover, C. reniformis collagen has been characterized and identified as mainly of type IV, thus promising application in epidermal regeneration strategies. In particular, its role on the selection of the rapid proliferating keratinocytes is addressed.

Combination of aloe vera improves the biological performance of chitosan membranes for skin tissue repair

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In the last years, aloe vera (AV), a tropical plant belonging to the liliaceae family, has gained popularity for medical, cosmetic and nutritional purposes. Commercially, AV appears in food supplements, as additive to drinks, coating fruits, moisturizer, and healing agent in cosmetic products, among others uses. The interest in AV comes from its chemical diversity and biological properties, which includes analgesic, anti-inflammatory, wound healing, antibacterial, immune modulation and anti-tumor activities. Therefore, the use of the therapeutic properties of AV gel could be very useful in the creation of materials for regenerative medicine. In the present work, the biomedical potential of systems composed by AV gel and chitosan (Cht), a natural polysaccharide, was assessed by processing them into membranes by solvent casting. Additionally, the blended system was crosslinked with genipin, a natural crosslinker agent. The crosslinking will bring the possibility to control the leaching out of AV gel portion from the blended membranes. The developed blended membranes were characterized by rough surfaces and controllable degradation behavior. Besides, the non- and crosslinked blended membranes have an increased stiffness and viscoelastic behavior as compared to chitosan membranes. Thus, these membranes will facilitate the penetration of the AV gel onto the skin, and the membrane may act at different stages of the healing process, while protecting the injury from infection. Additionally, chitosan will increase the stability of the polysaccharides and/or compounds present in the AV composition, which can in turn keep their natural biological activity. To examine cell behavior in the developed membranes, human dermal fibroblasts (hDFs) were seeded in the membranes. The results have shown that the blended membranes were more favorable for hDFs adhesion and proliferation in comparison with chitosan membranes. Furthermore, the calcein-AM results are in agreement with the findings obtained from the alamar blue assay (cellular viability) and DNA quantification (cell proliferation), suggesting that they are viable independently of the cell number present on the membrane surface. All findings indicated that the produced blended membranes present adequate properties for skin applications, namely as wound dressing materials.
Starch-based blends present an enormous potential to be widely used in the biomedical area, because they are totally biodegradable, inexpensive, available in large quantities. However, natural-based polymers have great limitations in processability particularly due to their usually high crystallinity which limits their solubility. This can be overcome by the use of ionic liquids which are recognized as ‘green’ replacements for conventional organic solvents. Earlier reports emphasized the use of certain ionic liquids to solubilize some natural macromolecules such as cellulose, starch, chitin, chitosan and silk fibroin. Furthermore, they present unique physicochemical properties, namely lower vapour pressure, excellent chemical and thermal stabilities, high ionic conductivity and easy recyclability. Starch based materials have been proceed in a variety of different morphologies and shapes by a number of different processes. In this work, starch/cellulose acetate (SCA) was dissolved in 1-butyl-3-imidazolium acetate, followed by regeneration of the polymer in different non-solvents (water, ethanol and isopropanol) in order to obtain membranes. Different concentrations of SCA (5 and 10%) in ionic liquid and drying techniques (vacuum oven and freeze drying) were studied. The starch/cellulose acetate structures were evaluated by their swelling capability, degradation behaviour and morphological features. Moreover, the influence of thickness on physical chemical properties of the membranes was assessed. The results revealed that membranes with lower thickness showed high water absorption, which by its turn accelerated their degradation rate. Furthermore, the membranes dried by vacuum oven present a more compact structure as compared those prepared by freeze drying. Some previous works reported SCA as a suitable material for tissue engineering purposes, supporting the cell adhesion. Then, in vitro cell culturing assays will be performed using osteoblast like cells (SaOs-2) and mouse fibroblast-like cell line (L929). The cell viability and proliferation on membranes will be evaluated through the MTS test and the DNA quantification. The development of innovative technology such as novel natural polymers materials is of greater interest in medical field. All findings suggested that the obtained structures (membranes) present adequate properties for several biomedical applications for instance drug delivery, skin substrates, guided bone regeneration or as coatings for medical devices.

**TS56**
Sustained release of prednisone and mesalamine from diatom exoskeletons: bioinspiration for the development of safe oral drug delivery devices to tackle gastrointestinal diseases

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Mesoporous silicon and silica-based particles have recently been synthesized and proposed for the controlled delivery of several drugs [1,2]. On the other hand, nature and in particular marine organisms have been the source and inspiration for the development of different biomedical applications, including drug delivery devices [3]. On the border of both rests diatoms exoskeletons, nature-made porous silica-based microparticles with amazing morphological features, promising a high potential in drug delivery. Nevertheless, its safety and drug permeability on oral formulations have not yet been studied. In this study, we have demonstrated that diatoms silica microparticles (DSM) have almost no toxicity in colon cancer cells Caco-2, HT-29, HCT-116 and Caco-2/HT-29, even at concentrations as high as 1000 μg/mL. Moreover, the delivery profile of two common drugs to address gastrointestinal diseases, mesalamine (anti-inflammatory) and prednisone (glucocorticosteroid). DSMs are able to release prednisone in a controlled manner and change its absorption pattern, which may improve the safety of its administration. In addition, DSMs can enhance the permeation of mesalamine. These results confirm the potential of DSMs for the development of oral formulations for the therapy of gastrointestinal diseases.

**References:**
Efforts in biomolecular nanotechnology are increasingly directed towards structural control of supramolecular self-assembly. Developments in this area of research are often inspired by living systems. Peptide-based self-assembly is attracting attention in mimicking intra- and extra-cellular fibrous networks with minimal complexity. It was previously found that using Fmoc-dipeptide gelators creates hydrogel scaffolds that are too hydrophobic for certain types of cells. Co-assembly of Fmoc-dipeptide fibres with a surfactant-like peptide derivative provides a handle for introducing chemical functionality, giving rise to more hydrophilic fibre surfaces that may contain functional peptides into the hydrogels. In this study, we demonstrated a facile supramolecular approach for the formation of functionalized nanofibres by combining the advantages of biocatalytic self-assembly and surfactant/gelator co-assembly. This is achieved by enzymatically triggered reconfiguration of free flowing micellar aggregates of pre-gelator (Fmoc-Fyp) and functional surfactants Fmoc-X (X = S, T or RGD, where RGD is a well-known cell adhesion motif) to form nanofibres that become coated with the surfactants. This results in the formation of fibres that display the functionality at the surface. Furthermore, by varying enzyme concentration, the gel stiffness and supramolecular organization of building blocks can be varied. Next step would be functionalization of peptide fibers with sugar-amphiphiles (e.g. Fmoc-Galactosamine) followed by testing their ability to bind to certain carbohydrate-binding proteins (e.g. lectin). These results would give an indication on the ability of these scaffolds to bind to certain types of cells (e.g. hepatocytes).

References:

**TS57**
Peptide-amphiphiles functionalization via co-assembly for cell culture

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Efforts in biomolecular nanotechnology are increasingly directed towards structural control of supramolecular self-assembly. Developments in this area of research are often inspired by living systems. Peptide-based self-assembly is attracting attention in mimicking intra- and extra-cellular fibrous networks with minimal complexity. It was previously found that using Fmoc-dipeptide gelators creates hydrogel scaffolds that are too hydrophobic for certain types of cells. Co-assembly of Fmoc-dipeptide fibres with a surfactant-like peptide derivative provides a handle for introducing chemical functionality, giving rise to more hydrophilic fibre surfaces that may contain functional peptides into the hydrogels. In this study, we demonstrated a facile supramolecular approach for the formation of functionalized nanofibres by combining the advantages of biocatalytic self-assembly and surfactant/gelator co-assembly. This is achieved by enzymatically triggered reconfiguration of free flowing micellar aggregates of pre-gelator (Fmoc-Fyp) and functional surfactants Fmoc-X (X = S, T or RGD, where RGD is a well-known cell adhesion motif) to form nanofibres that become coated with the surfactants. This results in the formation of fibres that display the functionality at the surface. Furthermore, by varying enzyme concentration, the gel stiffness and supramolecular organization of building blocks can be varied. Next step would be functionalization of peptide fibers with sugar-amphiphiles (e.g. Fmoc-Galactosamine) followed by testing their ability to bind to certain carbohydrate-binding proteins (e.g. lectin). These results would give an indication on the ability of these scaffolds to bind to certain types of cells (e.g. hepatocytes).

References:

**TS58**
Influence of the surface chemistry in the osteogenic activity of silica nanoparticles

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Polyelectrolyte multilayers (PEM) have been extensively studied in the biomedical field, namely as biodegradable systems, biosensors and drug delivery systems [1]. Using PEM films, it is possible to control the early steps in cell adhesion but also processes that occur at a longer time scale, such as cell differentiation and tissue formation. In this work, we study the influence of silica particles concentration, coated with polyelectrolytes, and we evaluate their biological activity (osteogenic differentiation of human bone marrow stem cells - hBMSC) as a function of the nanoparticle’s concentration and surface coating. We chose to execute the polyelectrolyte constructions using poly-L-lysine (PLL), a polycation which is widely used in cell culture to promote cell adhesion to solid substrates [2], and hyaluronic acid (HA), a polyanion, which is known for providing excellent biocompatibility and have been used for ophthalmic surgery, treatment of arthritis, drug delivery, and tissue engineering [3]. In this context, we prepared silica nanoparticles (diameter of ~174 nm) and silica particles coated with PLL-HA (diameter of ~200 nm). Further on, we studied the activity of these systems, under different concentration of particles (50 µg/mL, 25 µg/mL and 12.5 µg/mL), towards the osteogenic differentiation of hBMSC. The cell viability, cell proliferation, protein quantification (i.e. ALP DNA and MTS) and gene expression (evaluated by RT-PCR, i.e. Osteocalcin, Bone Sialoprotein, Runx2, Osteopontin and Osterix) was monitored, during 21 days. Our data indicates the overexpression of some of the osteogenic transcripts (e.g. Bone Sialoprotein, Osteocalcin and Osterix) in the hBMSCs cultured in the presence of SiO2-PLL-HA, under concentrations of 100 µg/mL and 50 µg/mL, in comparison with non-coated silica nanoparticles.

References:
**TS59**

Internalization, intracellular retention and release of drug-loaded dendrimer nanoparticles in astrocytes: patch-clamp electrophysiology and live confocal imaging studies

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Nanoparticles are one of the promising solutions for the current dilemma of drug transport to the central nervous system (CNS). The efficiency and specificity of treatments are compromised by the presence of cellular barriers that limit the passage of substances to the nervous tissue. Nanoparticles, and particularly dendrimers, are emerging as very promising tools for biomedical applications and envisioned as key players that will most likely have major roles in improving the means of diagnostics, imaging and therapeutics. However, despite the tremendous amount of literature involving NP synthesis, functionalization and in vitro and in vivo evaluation, basic knowledge on the interactions of these materials with the living systems is still sparse. The mechanisms of cellular internalization and clearance, intracellular trafficking and distribution, as well as degradation, remain unexplored. Yet, herein we investigated the interaction of dendrimer nanoparticles with astrocyte membranes using patch-clamp electrophysiology capacitance quantification and live confocal imaging. Carboxymethylchitosan/ poly(amide)amine (CMCht/PAMAM) dendrimer nanoparticles were developed recently in our lab and loaded with the corticosteroid methylprednisolone (MP). These dendrimer NP recently revealed a remarkable potential for administration and diffusion in the CNS, having inclusively showed interesting therapeutical properties in animal models of injury. In this study, we investigated how the NP interfered with the frequency of astrocyte membranar events, such as endocytosis and exocytosis vesicle formation. Primary astrocytic cultures were incubated with NP and: (i) prepared for electrophysiological readings; or (ii) labeled for endocytotic or exocytotic vesicles, for live confocal imaging. The patch clamp electrophysiology data revealed differences in the frequency of events arising in the astrocyte membrane in the presence of the nanoparticles, indicating that they interfere with the formation/fusion of vesicles, both endocytic and exocytotic. These alterations were more pronounced and significant regarding the formation of exocytotic vesicles. The live confocal imaging of both these types of vesicles confirmed that the nanoparticle trafficking in astrocytes involves these pathways, since co-localization of nanoparticles was seen both with endocytotic and exocytotic vesicles. We are directly showing for the first time that the MP-loaded CMCht/PAMAM dendrimer nanoparticles enter the endosomes and exosomes of astrocytes, meaning that after entering the cell the NP associate with exocytic vesicles to follow the exit route and be cleared from astrocytes. Nonetheless, the MP-loaded dendrimer NPs remain in the cells for a sufficient time period (about a week) to allow therapeutic intervention, while exiting the cells via exocytosis, in a dynamic way.

**TS60**

Adhesion of adipose-derived mesenchymal stem cells to patterned protein-glycosaminoglycan surfaces

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Proteins and glycosaminoglycans (GAGs) are the main constituents of the extracellular matrix (ECM). Until very recently, GAGs were considered as pure structural components while proteins have been associated with crucial cell signalling processes. Nowadays, it is well accepted that these two ECM components act in synergy and are equally critical for the development, growth, function or survival of an organism. In this work, we have developed surfaces that display these two classes of biomacromolecules in spatially controlled fashion. Sulfated GAGs and hyaluronic acid were covalently bound to amino functionalised surfaces and proteins were patterned by micro-contact printing on top of the GAGs. Among proteins, we have selected albumin as a small non-adhesive molecule and fibronectin as a larger, adhesive protein which also has heparin-binding domains. Adipose-derived stem cells (ADSC) were studied in contact with those surfaces. We found that ADSC adhere on the glycan pattern when albumin was used as a model protein and the adhesion and cellular morphology do not depend on the immobilised GAG. Moreover, the cells were positive for CD44. When fibronectin was used instead, the cells were found on the protein pattern where they form large cytoskeleton with well structured actin fibers. We did not find CD44 positive cells for those surfaces.
Double entrapment of VEGF by PCL nanoparticles loaded into polyelectrolyte multilayer films

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Delivery of growth factors and control of vascularization are prominent problems in regenerative medicine. Vascular endothelial growth factor (VEGF) has been used both in vitro and in vivo for promotion of angiogenesis but due to its short half-life its controlled delivery is a sought after method. In this study we present a new concept composed of degradable drug loaded nanoparticles entrapped into exponentially growing multilayer films. Through hydrolysis of the nanoparticles, the drug can be delivered over long periods in a controlled manner. Poly(L-caprolactone) nanoparticles (460 nm) were loaded with VEGF and in turn the release of VEGF from a surface is controlled by a thick layer-by-layer polyelectrolyte film (~5 μm). Direct loading of VEGF inside the film was not efficient for a long-term purpose. When VEGF loaded nanoparticles were introduced into the film, the particles were equally distributed inside and were stable after several washes. Moreover, presence of the film sustained the release of VEGF for 7 days. Addition of the nanoparticles to the film promoted endothelial cell proliferation, mainly due to the presence of VEGF. Mechanical properties of the film (Young moduli) were also improved by the presence of nanoparticles. However in the presence of the film loaded with nanoparticles and without any direct contact with this film, endothelial cell growth was also enhanced on polystyrene and on Transwell insert surfaces which demonstrates the effectiveness of the nanoparticles not only to improve mechanical properties of the film but also to deliver active VEGF. Increase in nitric oxide levels as an indicator of endothelial cell activity was monitored and was related to the release of VEGF from nanoparticle/film system. Finally, such a system can be used as an auxiliary delivery body within implants to finely control the release of bioactive agent containing nanoparticles.
WORKSHOP ABSTRACTS

Nanosystems for Medicine: fundamentals, synthesis & application

7 | 9 October 2012
Porto Palácio Congress Hotel & Spa, Porto, Portugal
www.termstem.org

Conference Chair:
Rui L. Reis

Organized by:
3B’s Research Group, University of Minho, Braga, Portugal
www.3bs.uminho.pt
WORKSHOP HIGHLIGHTS

TERM STEM 2013 will be organised back to back with POLARIS 1st Workshop that will take place from 7 to 9 October 2013, in Porto, having the same venue the Porto Palácio Congress Hotel & Spa.

This workshop is being organized on the scope of the European FP7 REGPOT project entitled POLARIS: Unlocking the research potential of 3B’s Research Group, University of Minho, in Nanomedicine field to strengthen its competitive position at the European level.

This 1st workshop will focus the materials structuring at the nanoscale emphasising NanoMedicine as an application field. This is a very restricted event (about 50 attendees) that targets strengthening the research potential of a specific region and in specific research topic.

Scientific Topics:
- BioNanoMaterials for Regenerative Medicine
- Self-organization at the Nanoscale
- Targeted Delivery using Nanotechnology

Looking forward to meeting you in Porto!

Rui L. Reis
The Conference Chair
Keynote Lectures

LP01
Tuning protein designs to modulate material properties
DL Kaplan
Department of Biomedical Engineering, Tufts University, Boston, Massachusetts 02155 USA

Tailored biomaterials with tunable functional properties are desirable for many needs ranging from medical devices to scaffolding in tissue engineering and regenerative medicine and for drug delivery. To achieve increased predictability of biomaterials functionality, multiple parameters in polymer design need to be considered. Along with such factors, appropriate models must be available that can be used to engineer tailored material solutions, including at different scales of structural hierarchy as they relate to function. The importance of multiscale engineering and the combined use of modeling and experiment in advancing designs of biomaterials will be the focus of the talk. This is an emerging field where modeling can inform polymer design and vice versa. The approaches being pursued in protein-polymer design and engineering, processing and modeling/predictions of function will be described as an example on how this approach can be used to broaden predictive structure-function relationships in the field.

LP02
From supramolecular chemistry to nanofabrication
DN Reinhoudt
Laboratory for Supramolecular Chemistry and Technology, MESA+ Institute for Nanotechnology, University of Twente, The Netherlands

Like in microelectronics, the fabrication of nanomaterials and devices will most likely start with the patterning of surfaces, at the nanoscale. Individual nanostructures can be manufactured most easily when confined to a surface. We have developed host-guest systems that make use of supramolecular chemistry in water. Nanopatterning on self-assembled monolayers of beta-cyclodextrine receptors (molecular printboards) can be achieved by supramolecular nano-imprint lithography or DPN nanolithography. Using the concept of multivalency, molecules can be anchored permanently or in a dynamic regime. Several examples of the immobilization of biomolecules at such surfaces will be discussed. A second way to pattern surfaces uses dynamic covalent chemistry. This methodology has the same advantages as supramolecular patterning, but can easily be combined with irreversible confinement, e.g., by reduction of an imine linker. We will discuss the patterning of surfaces with proteins that selectively recognize cancer cells. In this work we have discovered that under conditions of μCP or DPN lithography covalent reactions are much faster than from solution. This observation has been used in click chemistry at surfaces and applied for the generation of DNA arrays.

LP03
Self-assembly of peptide building blocks at the nano-scale: mechanism of association and technological applications
Prof. Dr. E Gazit
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The formation of ordered amyloid fibrils is the hallmark of several diseases of unrelated origin. In spite of grave clinical consequence, the mechanism of amyloid formation is not fully understood. We have suggested, based on experimental and bioinformatic analysis, that aromatic interactions may provide energetic contribution as well as order and directionality in the molecular-recognition and self-association processes that lead to the formation of these assemblies. This is in line with the well-known central role of aromatic-stacking interactions in self-assembly processes. Our works on the mechanism of aromatic peptide self-assembly, lead to the discovery that the diphenylalanine recognition motif self-assembles into peptide nanotubes with a remarkable persistence length. Other aromatic homodipeptides could self-assemble in nano-spheres, nano-plates, nano-fibrils and hydrogels with nano-scale order. We demonstrated that the peptide nanostructures have unique chemical, physical and mechanical properties including ultra-rigidity as aramides, semi-conductive, piezoelectric and non-linear optic properties. We also demonstrated the ability to use these peptide nanostructures as casting mould for the fabrication of metallic nano-wires and coaxial nano-cables. The application of the nanostructures was demonstrated in various fields including electrochemical biosensors, tissue engineering, and molecular imaging. Finally, we had developed ways for depositing of the peptide nanostructures and their organization. We had use inkjet technology as well as vapour deposition methods to coat surface and from the peptide “nano-forests”. We recently demonstrated that even a single phenylalanine amino-acid can form well-ordered fibrilar assemblies of distinct electron diffraction pattern and toxic properties.
Sonochemistry is an excellent technique to coat functional nanomaterials on various substrates, and imparting new properties to the substrates. After a short demonstration of coating NPs on ceramics and stainless steel, I’ll present the coating of textiles such as polyester, cotton, nylon, and nonwoven. In all cases a homogeneous coating of NPs was achieved. Silver is known for generations as antibacterial, and indeed the Ag NPs have killed the gram-negative E. Coli (strain 1313) as well as the gram-positive Staphylococcus aureus (strain 195) bacteria very efficiently. Later, since the FDA shows less enthusiasm towards nanoAg we have moved to NPs of ZnO, CuO and MgO as antibacterial agents. They were coated on the above-mentioned fabrics and showed excellent antibacterial properties. The coated textiles were examined for the changes in the mechanical strength of the fabric. A special attention was dedicated to the question whether the NPs are leaching off the fabric when washed repeatedly. The coated ZnO NPs on cotton underwent 65 washing cycles at 92 °C in water in a Hospital washing machine, no NPs were found in the washing solution and an the antibacterial behavior was maintained. Our vision is that all the textiles in the Hospitals of the future will coated by antibacterial NPs. The mechanism of killing the bacteria was studies and will be presented. Our research in the last few months was directed towards finding nanomaterials that can kill resistant bacteria. I will present recent results on NPs of antibiotic materials, as well as inorganic NPs that can do this job.

Polymeric nanoparticles have found application in varied fields including drug delivery and medical imaging. Particle’s properties have a significant impact on their therapeutic performance including circulation half-life, drug release rates and toxicity. Recent studies have shown that particle morphology plays a significant role in determining the biological and therapeutic outcome of nanoparticles. My talk will focus on discussing some of the key outcomes of nanoparticles that can be controlled through engineering particle morphologies. We have devised methods to generate particles of several distinct morphologies and studied their impact on key processes in drug delivery including phagocytosis, circulation, adhesion of vascular walls, and targeting. Based on this understanding, we have designed novel particles that demonstrate enhanced targeting. Our studies demonstrate that particle shape provides a new dimension in engineering of polymeric carriers and opens up new opportunities in drug delivery. In addition to shape, we demonstrate that controlling mechanical properties of carriers also offers unique opportunities. Specifically, we have synthesized flexible particles made from proteins that mimic the physical and functional properties of body’s own circulating cells such as red blood cells. Particles that mimic the size, shape and flexibility of natural circulating cells offer advantages that are typically lacking in conventional spherical polymeric particles. The motivation to use physical properties of nanoparticles to control biological function is provided by the biology itself. In nature, numerous examples can be found where physical aspects, such as shape, mechanical properties and compartmentalization are crucial to biological function. Physical attributes such as size, shape and mechanical properties form essential building blocks of biology. This realization forms the basis of the new paradigm in design of nanoparticles.

From the perspective of thousands of years of history, the role of biomaterials in healthcare and wellbeing of humans is at best accidental. However, since 1970s with the introduction of national regulatory frameworks for medical devices, the biomaterials field evolved and reinforced with strong science and engineering understandings. The biomaterials field also flourished on the backdrop of growing need for better medical devices and medical treatments, and sustained investments in research and development. It is estimated that the world market size for medical devices is ~300 billion dollars and for biomaterials it is ~30 billion dollars. Healthcare is now one of the fastest growing sectors worldwide. Legions of scientists, engineers, and clinicians worldwide are attempting to design and develop newer medical treatments involving tissue engineering, regenerative medicine, nanotech enabled drug delivery, and stem cells. They are also engineering ex-vivo tissues and disease models to evaluate therapeutic drugs, biomolecules, and medical treatments. Engineered nanoparticles and nanofiber scaffolds have emerged as important class of biomaterials as many see them as necessary in creating suitable biomimetic micro-environment for engineering and regeneration of various tissues, expansion & differentiation of stem cells, site specific controlled delivery of biomolecules & drugs, and faster & accurate diagnostics. This lecture will capture the progress made thus far in pre-clinical and clinical studies. Further this lecture will discuss the way forward for translation of bench side research into the bed side practice. This lecture also seeks to identify newer opportunities for biomaterials beyond the medical devices.
LP07  
**Biotic self-assembling systems for polymer therapeutics**  
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The design and development of “Polymer Drugs” are one of the most attractive fields of advanced systems for new therapeutic applications of bioactive compounds. In one biomimetic approach, these systems are based on the preparation of polymeric chains with specific hydrophobic or hydrophilic character by the reversible linking of bioactive compounds to macromolecular systems. This character is achieved by the chemical reaction of specific functional groups present in the macromolecule or by copolymerization of functionalized bioactive compounds or drugs, with specific polymerizable functions. This approach allows very interesting designs by selecting polymerization mechanisms and composition of the active monomers, to give high molecular weight polymers with controlled microstructure and compositions. Copolymer systems based on the adequate composition of hydrophobic and hydrophilic sequences present a high tendency to form self-assembling nanoparticles that can be used for the targeting and release of toxic bioactive components. A new family of acrylic polymers bearing sequences of acrylic derivatives of 5-amiononaphthalen sulphonic acid will be presented as systems that are able to interact with growth factors (FGF and VEGF) and therefore to present a very interesting inhibition of angiogenic processes at relatively low dose and very low toxicity. The behavior of these systems has been tested in vitro using a 3D model for cell cultures, as well as in vivo in an animal model with good correlation of results. Two different families of polymer systems will be presented with different composition and morphology. They are based on copolymers prepared by the free radical polymerization of functionalised acrylic monomers and vinyl compounds with very different reactivity ratios. The average composition of systems prepared at high conversion gives self-assembling polymers with specific morphologies and very different behaviour depending on the distribution of monomeric sequences, but with very low toxicity which allows the application as highly active antitumoral and antiangiogenic compounds that can be applied by local injection in the human body.


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Biological systems commonly serve as inspiration for the development of new materials with properties that are not normally associated with man-made systems. These properties include multi-functionality, adaptive mechanical properties and reconfiguration in response to applied stimuli. These biological systems are however complex, fragile and often not completely understood. It is our vision to develop new molecular technologies that are inspired by the materials found in living systems, but that are accessible to chemists and may be exploited in technological and biomedical applications. In order to achieve this, we use a concept of ‘minimal biology’ where each component is as simple as possible but retains its function. In this talk, our latest research results in this area will be described, focusing on the use of simple self-assembling systems based on aromatic peptide amphiphiles. We will demonstrate the control of (i) nanoscale morphology through peptide design (Figure), (ii) functionality by using co-assembly approaches, (iii) mechanical properties and its use as tissue mimics, (iv) responsiveness to salts and finally our very recent work on (v) responsiveness to audible sound. Applications will be discussed in cell culture and in the interfacing of biology with electronics.

References

Nanoscale interface between engineered matter, and living organisms: understanding the biological identity of nanosized materials and implications for nanomedicine

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Nanoscale materials can interact with living organisms in a qualitatively different manner than small molecules. Crucially, biological phenomena such as immune clearance, cellular uptake and biological barrier crossing are all determined by processes on the nanometer scale. Harnessing these endogenous biological processes (for example in creation of new nanomedicines or nanodiagnostics) will therefore require us to work on the nanoscale. This ensures that nanoscience, biology and medicine will be intimately connected for generations to come, and may well provide the best hope of tackling currently intractable diseases.

These same scientific observations lead to widespread concern about the potential safety of nanomaterials in general. Early unfocussed concerns have diminished, leaving a more disciplined and balanced scientific dialogue. In particular a growing interest in understanding the fundamental principles of bionanointeractions may offer insight into potential hazard, as well as the basis for therapeutic use.

Whilst nanoparticle size is important, the detailed nature of the nanoparticle interface is key to understanding interactions with living organisms. This interface may be quite complex, involving also adsorbed proteins from the biological fluid (blood, or other), leading to a ‘protein corona’ on the nanoparticle surface that determines its “biological identity”. We discuss how this corona is formed, how it is a determining feature in biological interactions, and indeed how in many cases can undermine efforts at targeting nanoparticles using simple grafting strategies. Thus, nanoparticle interactions with living organisms cannot be fully understood without explicitly accounting for the interactions with its surroundings, i.e. the nature of the corona.

References
Abstracts List

POLo1
Silica nanoparticles as dexamethasone delivery systems able to induce the osteogenic differentiation of human bone marrow-derived mesenchymal stem cells
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Bioactive glasses, especially silica-based materials, are reported to present osteoconductive and osteoinductive properties, fundamental characteristics in bone regeneration [1,2]. Additionally, dexamethasone (Dex) is one of the bioactive agents able to induce the osteogenic differentiation of mesenchymal stem cells by increasing the alkaline phosphatase activity, and the expression levels of Osteocalcin and Bone Sialoprotein [3]. Herein, we synthesised silica (SiO2) nanoparticles (that present inherent bioactivity and ability to act as a sustained drug delivery system), and coated their surface using poly-L-lysine (PLL) and hyaluronic acid (HA) using the layer-by-layer processing technique. Further on, we studied the influence of these new SiO2-polyelectrolyte coated nanoparticles as Dex sustained delivery systems. The SiO2 nanoparticles were loaded with Dex (SiO2-Dex) and coated with PLL and HA (SiO2-Dex-PLL-HA). Their Dex release profile was evaluated and a more sustained release was obtained with the SiO2-Dex-PLL-HA. All the particles were cultured with human bone marrow-derived mesenchymal stem cells (hBMSCs) under osteogenic differentiation culture conditions. hBMSCs adhered, proliferated and differentiated towards the osteogenic lineage in the presence of SiO2 (DLS 174nm), SiO2-Dex (DLS 175nm) and SiO2-Dex-PLL-HA (DLS 679nm). The presence of these materials induced the overexpression of osteogenic transcripts, namely of Osteocalcin, Bone Sialoprotein and Runx2. Scanning Electron Microscopy/Electron Dispersive Spectroscopy analysis demonstrated that hBMScs synthesised calcium phosphates when cultured with SiO2-Dex and SiO2-Dex-PLL-HA nanoparticles. These results indicate the potential use of these SiO2-polyelectrolytes coated nanoparticles as dexamethasone delivery systems capable of promoting osteogenic differentiation of hBMSCs.

References

POLo2
Cytotoxicity evaluation of hydroxyapatite-nanoparticles in vitro – finding a test-system to resemble the in vivo situation
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Biomaterials based on nano-particles have gained increased attention because of its potential application in several medical fields including tissue engineering (TE), dentistry and pharmacy. We aim to study the effects of nanoparticles suited for TE strategies on cells in vitro as well as in vivo. Ostim® is a nano-hydroxyapatite aqueous paste approved for clinical use as bone defect filling material. In a previous study in a bone defect model, Ostim® showed to support bone formation with no inflammatory reaction of the surrounding tissue [1]. Interestingly, the addition of the paste on cultured cell in a typical cytotoxicity test set-up resulted in a toxic effect in vitro. When exposing the cells to the particles in transwell-sytems, we were able to demonstrate that direct cell-particle contact leads to cytotoxicity, which was further analyzed by TEM. To optimize in vitro test systems for biomaterials to meet the in vivo situation, we embedded cells either in fibrin matrix prior to the addition of the particles or placed the material between two layers of viable human amnion. Subsequently, cell viability of the embedded cells or the cells of the amniotic membrane was quantified. Placing the paste between stretched amniotic layers showed results comparable to the in vivo situation. This test system presents a promising candidate for biomaterial evaluation.

**POLo3**

**Nanostructured and biofunctionalized water-in-oil droplets as tools for homing T cells**

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While the beneficial impact of modifying and/or targeting T lymphocytes is becoming increasingly accepted in the treatment of different diseases, the road towards cell-based immunotherapy is still long and winding. Major challenges that remain include, amongst others, the guidance and exquisite regulation of immune processes *ex vivo*. In part, this is due to the difficulties of simulating *ex vivo* the intimate cellular interactions that occur between T cells and antigen-presenting cells (APCs).

The presentation will cover a development and characterization of novel nano-structured and specifically biofunctionalized droplets of water-in-oil emulsions as 3-D APC analogues. To create the droplets, we have synthesized a new type of gold-linked surfactants and used a drop-based microfluidic device. The efficiency of the gold nanoparticles in the nanostructured droplets to provide the required chemical and biological key functions of the APC will be presented.

Combining flexible biofunctionalization with the pliable physical properties of the nanostructured droplets can play a crucial role as it results in a flexible and modular system that closely models in situ APC-T cell interactions. The ability of T cells to exert forces in all three dimensions on the biomolecules held by the drop may be important in evaluating the affinity and function of antigen receptors. Moreover, the ability to create a well-defined picoliter environment for T cell stimulation is preeminent for long-term monitoring of individual T cells over the course of their activation and differentiation and provides this system with superior properties in comparison to previously reported synthetic APC analogs.


**POLo4**

**Evaluation of targeted gene delivery to cancer cells through high throughput fluorescence microscopy**

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Nanomedicine holds enormous promise for the development of specialized delivery systems with unique characteristics that may utterly alter current medical practice. However, the tremendous potential of these nanosized carriers has, until nowadays, met with rather limited success, particularly in the area of cancer research. Interestingly, this fact is considered to be highly correlated with the lack of suitable pre-clinical testing models that can provide appropriate insights of the in vivo performance of the nanosized delivery systems. Therefore, addressing this issue assumes critical importance during nanoparticle manufacturing stages. In this context, the establishment of cell cultures that mimic the tumor microenvironment can provide further insights of the biological performance of targeted nanocarriers. Herein we used confocal laser scanning microscopy to elucidate the complex events underlying nanomedicine delivery in cancer cell cultures. The use of CLSM has demonstrated its remarkable potential as a pivotal tool to assist in the design of more efficient targeted delivery systems in the future.
**POLo5**

Silica-borate glasses for bone tissue engineering applications

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Commercial silicate-based bioglasses, including 45S5 glass have been subject of vast interest in the biomedical field for many years [1]. Several limitations of those commercial glasses, such as: limited degradability, low porosity and it generates an acidic medium that induce inflammatory response led to the development of silica-borate glass systems for biomedical applications [2-5]. Silica-borate systems can overcome some of the enumerated drawbacks, when used as part of composite systems. They can improve the strength of the composite through the reaction of the glass degradation products (e.g. carboxylic) with the polymeric matrices. Additionally, controlling the degradation it is possible to increase the porosity of composites.

The main objective of this work was to prepare silica-borate glass compositions by melt quenching. Glass compositions of general formula 0.20B\(_2\)O\(_3\):0.40SiO\(_2\):xMgO:yCaO:(0.35-x-y)SrO:0.05Na\(_2\)O (molar ratio, where x, y = 0.35 or 0.00, and x ≠ y) were synthesised, and composite fibres were prepared by wet spinning of a PLA solution containing a suspension of the glass particles. Fibre meshes of the processed fibres were prepared and their cytotoxicity was evaluated by direct contact with human osteosarcoma cell line Saos-2, throughout 7 days of incubation (37 °C and 5% CO\(_2\) atmosphere). For day 1, 3 and 7, cell proliferation (DNA quantification method) and metabolic activity (MTS method) were monitored. Additionally, it was tested the water uptake and weight loss of the developed systems, as well as their capacity to form a calcium phosphate layer when immersed in simulated body fluid (SBF).

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**POLo6**

Nanocarriers based on interpolyelectrolyte complexation of Sulphated polysaccharide-b-PEG diblock copolymers and PLL

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Glycosaminoglycans (GAGs) are integral part of the closest cellular environment: they can be found on the cells surface and in the extracellular matrix, where they interact with different proteins acting as local regulator of their activity. The use of GAGs in the preparation of protein delivery nanosystems is, therefore, prominent but so far, underexploited mainly because of the heterogeneity (composition and molecular weights) of natural glycans and the multistep procedures needed to obtain GAGs’ synthetic analogues and diblock copolymers.1 Recently, we have shown that oxime click reaction can be applied as a straightforward methodology for the synthesis of poly(ethylene glycol) (PEG)-hyaluronic acid (HA) diblock copolymers.2 These copolymers formed nanosized interpolyelectrolyte complexes (45 to 150 nm) by interaction with poly- L-lysine (PLL).3 Unfortunately, these complexes are not stable at physiological ionic strength. Herein, we describe a strategy to overcome this drawback; chondroitin sulphate-b-PEG diblock copolymers (CS-b-PEG) were obtained using the same oxime click reaction. The stronger negative charge of sulphate groups (versus the carboxilic groups present in HA) resulted in the complexes with higher stability: interpolyelectrolyte complexes between PLL and (CS-b-PEG) are stable up to 260 mM ionic strength. Because carbohydrates do not activate T-cells, we believe that the reported herein complexes have an enormous potential in both drug delivery and vaccination fields.4

Marine sponges – a new source of bioactive ceramics for tissue engineering and regenerative medicine applications

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Marine organisms are exceptionally rich in natural products and present huge prospective for biomedical applications. In this work we have studied the potential of bioactive ceramics from different sponge species, namely Petrosia ficiformis, Agelas oroides and Chondrosia reniformis, for novel biomedical applications. Studies reported in the literature have already demonstrated the potential of carbonate corals as a source of bioactive ceramics. However, similar studies directed towards the valorization of marine sponge skeletons are still missing. The bioactive ceramics, exempt of organic components, were obtained after calcination of the sponges at 750°C for 6 hours in a furnace. The powder was recovered and Scanning electron microscopy (SEM) was used to observe the morphology and gain insight of the elements spatial arrangement. Spectroscopic elemental analysis (EDS) was used to determine the chemical composition and has shown that Petrosia ficiformis skeleton is constituted mainly by silicate, while Chondrosia reniformis spicules are mostly calcium carbones. On the other hand, the ceramics obtained from Agelas oroides present a combination of silicate and calcium salts.

In vitro bioactivity of the bioactive ceramics was evaluated in simulated body fluid (SBF), after 3, 7 and 14 days of incubation. Observation of the bioactive ceramics by SEM, coupled with EDS, has shown that it is possible to induce the precipitation of calcium-phosphate crystals, consistent with the deposition of HA. Cytotoxicity of developed bioactive ceramics has also been assessed, comparing their behaviour with synthetic biomaterials. Results obtained thus far have shown the potential of bioactive ceramics from marine sponges for its use in biomedical applications, namely in tissue regeneration approaches.

Development of new chimeric proteins for tissue engineering

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Tissue engineering is an emerging field focused on the development of novel bioactive multifunctional materials that can be used to replace damaged and failing tissues. However, these biomaterials often present several problems such as loss of mechanical and/or biological properties and adverse immune responses. The use of natural polymers, such as proteins, provides a promising solution for these drawbacks. With advances in recombinant DNA technology and biotechnology, it is possible to design and produce new materials with different features by combining domains of different proteins in the same fusion protein. Spider dragline silk proteins have been suggested to have a large potential for many different biomedical applications due to its outstanding mechanical properties. In addition, spider silk is also biocompatible, hypoallergenic and completely biodegradable.

Recently, silk copolymers based on repeats of the consensus sequence of MaSp1 (major ampullate spidroin I) from Nephila clavipes (6mer) have been fused with different functional proteins, peptides and protein motifs, showing promising results [1, 2]. In this project, by exploring the use of recombinant DNA techniques, we have constructed new silk copolymers composed of a structural motif (6mer) fused with functional domains namely GFOGER (from collagen type I) and FNI (fibronectin domain II); both are involved in cell adhesion and angiogenesis processes which are key factors in tissue engineering. Expression and purification of the new chimeric proteins were successfully attained in Escherichia coli by means of auto-induction media. Furthermore, formic acid can be explored as a solvent for processing of the aforementioned recombinant copolymers. Results from the characterization of these biomaterials will be presented.
**POL09**

A, possible, molecular cause for carbon nanomaterials increased toxicity

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Carbon nanomaterials have been proposed as nano-vehicles to deliver genetic or therapeutic material into the interior of cells because of their capacity to transpose cell membranes. A detailed picture of the molecular mode of action of such a delivery is, however, difficult to obtain because of the concealing effects of the cell membrane. We will discuss our previous and present computational studies of membrane insertion, at the molecular level, of different carbon nanomaterials. The following materials will be addressed: individual and bundled carbon nanotubes, graphene flakes and fullerenes, ranging from the C60 to carbon nano-onions. Finally, we will discuss how fundamental molecular interactions are, likely, to blame for the large toxicity observed in some carbon materials.

**POL10**

Drug-layered double hydroxides nano-hybrids: role of layered double hydroxides as nano-reservoir and nano-vehicle

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Drug delivery has attracted great attention due to their clinical, medical and wide range of technical applications. A large number of drug deliveries with various bioavailabilities have been discovered but the potential for pharmaceutical prospects are still limited. Thus development of drug delivery with safe and controlled bioavailability is of great interest due to medical point of view. Recently, increasing attention has been directed the development of bio-inorganic hybrid systems in the drug delivery for the controlled release of biochemicals. Bio-inorganic systems can allow safe and controlled delivery of various bioagents into targets with high efficiency. In this proposal, we will make an attempt to synthesis layered double hydroxide (LDH) nanoparticles and further develop innovative nano-drug deliveries by intercalation of various drugs into the interlayer of LDH. Both LDH and drug-LDH nanohybrids will characterize by using field emission scanning electron microscopy (FESEM), energy dispersive spectroscopy (EDS), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and UV-visible absorption spectroscopy. The drug contents will study by thermogravimetric analysis (TGA) and elemental analysis (CHN). Drug-LDH nano-hybrid will apply in a formulation for the oral administration and the drugs release profile will study by simple deintercalation of drugs by various anions using UV-visible and HPLC.
**POL11**  
Segmental characterization of the cellular density of human knee meniscus  
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Meniscus is a fibrocartilaginous tissue that has an important role in biomechanics of the knee joint. Fibrochondrocytes and fibroblast-like cells are the two main cell populations present in the meniscus. Meniscus is distinguished into two regions: avascular and vascular region. Cellularity varies within the human meniscus, specifically between avascular and vascular regions of the meniscus, but also between anterior, medial and posterior parts. Cellularity is one of the important characteristics that should be considered in tissue engineering and regenerative medicine strategies. The aim of this study is to calculate the 3D cell density of human meniscus using histological slides. Meniscus tissues obtained from donors are prepared into Giemsa stained histological slices with a thickness of 30 μm. Slices are grouped by their anatomical location into three parts: anterior, medial and posterior. Cells in the defined areas of avascular and vascular regions are counted either as fibrochondrocytes or as non-fibrochondrocytes using a stereomicroscope. 3D cell densities of different region and parts of the meniscus are estimated by calculating the number of the cells found in unit volume. The initial results show that the 3D cell density is around 8000 cells/mm3 in vascular part that is the almost double of the density in avascular part. Chondrocytes take up more than the half of the total cell amount in avascular part, and less than the half in the vascular part. This work aims to contribute to the knowledge of cellularity of human meniscus and facilitates the development of more efficient strategies for meniscus tissue engineering. The authors thank the financial support of the MultiScale-Human project (Contract number: MRTN-CT-2011-289897) in the Marie Curie Actions—Initial Training Networks.

**POL12**  
Immunization of bioactive factor-loaded liposomes at the surface of electrospun nanofibers targeting tissue engineering strategies  
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The ability to manipulate and control the surface properties is of crucial importance in the designing of scaffolds for Tissue Engineering (TE) and Regenerative Medicine. Electrospun nanofibers (NFM), due to their morphology and fibrous structure have received much attention as potential biomedical devices, TE scaffolds and drug delivery carriers. Liposomes, a nanoparticle release system made by physiological material (phospholipids), hold tremendous promise as release systems. Liposomes may be combined with scaffolds to maintain a sustained and local delivery of the loaded drugs. The main objective of the present study is to evaluate the efficacy of dexamethasone (Dex) loaded liposomes immobilized on the surface of polycaprolactone (PCL) electrospun nanofiber meshes (NFM) as release system, for the induction of the osteogenic differentiation of human bone marrow-derived mesenchymal stem cells (hBMSCs). The PCL NFM surfaces were activated to insert amine groups onto the NFM surfaces. Afterwards, SH groups were inserted at the surface of the NFMs through the reaction of the aminated surfaces with 2-iminothiolane. Ellman’s reagent method was used to quantify the SH groups onto the NFM surfaces. Dex-loaded liposomes were covalently immobilized at the surface of chemically functionalized electrospun PCL NFMs. The in vitro release profile demonstrated a sustained release of Dex during 21 days, after an initial burst release of 12 h. Biological assays showed that Dex-loaded liposomes immobilized at the surface of electrospun PCL NFMs did not exhibit any cytotoxic effect, promoting the osteogenic differentiation of hBMSCs. We herein validate the concept of using liposomes immobilized at the surface of a nanostructured fibrous system to be used as an advanced cell carrier device with autonomous release of growth/differentiation factors relevant for tissue engineering and regenerative medicine strategies.
POL13
PCL nanofibers with magnetic nanoparticles for mesenchymal stem cell proliferation
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Nowadays magnetic nanoparticles have a great potential for biomedical applications due to their specific properties. These particles usually consist of magnetic component, often magnetite (Fe3O4) or other iron oxide, and the nonmagnetic component which ensure interaction with biological material. Magnetic particles have an important role especially for the separation of biomolecules and diagnostics, however in recent years, there are also applications of magnetic nanoparticles in the field of tissue engineering. Mesenchymal stem cells are unique especially for their ability to differentiate into many cell types and are thus very promising tool for tissue regeneration. However, there are problems with their insufficient quantity, so it is necessary to expand these cells in vitro. Herein, nanofibers were created by electrospinning from a mixture of polycaprolactone and magnetic particles (Fe3O4) at size of 50 nm. Viability and proliferation of porcine mesenchymal stem cells as well as alkaline phosphatase activity were monitored at 1st, 7th and 21st day after seeding. Adhesion and proliferation of the cells were also verified by confocal microscopy. Significantly better viability and proliferation of cells in the presence of magnetic nanoparticles were demonstrated by MTS and PicoGreen assays, respectively. These measurements also correlate with the results of confocal microscopy. Alkaline phosphatase activity of mesenchymal stem cells was also increased in vitro. The double overexpression of caspase-3 gene was detected in first days and followed by significant differences in necrosis marker (CASP3), necrotic (HMGB1) and hypoxic (HYOU1) markers, and cell adhesion molecules (CDH2) were evaluated through RT-qPCR assay. Results: Live/Dead assay shows cells stay alive during the whole experimental time (7 days), however undergo proliferation down-regulation (PicoGreen DNA quantification assay). RT-qPCR shows 20-60 times increase in HSP70B expression. Double overexpression of caspase-3 gene was detected in first days, and followed by it’s expression stabilization. There was not found significant differences in necrosis marker (HMGB1) expression, neither in hypoxia marker (HYOU1). Double up-regulation of adherence molecule expression (cadherin-2) was detected in first days and followed by significant down-regulation of cadherin-2. Conclusions: The process of EHDJ encapsulation was found has no cytotoxic effect on cells viability. However, it leads to cell stress accumulation, and induces HSP70B overexpression, that in turn induces cell stress resisting and cells recovering. In fact, it was seen the absence of stress markers expression -apoptotic, necrotic and hypoxic, according to the literature data. Under HSP70B overexpressed conditions different types of cells are protected against stresses such as heat, apoptotic markers or hypoxia [3].

References:
A critical component for successfully engineering complex 3D tissue from a cell source is the production and utilisation of the appropriate 3D scaffold. Cells seeded on a flat surface grow typically in a monolayer fashion, while 3D cell cultures can only be achieved via their growth in a 3D micro-environment. The success of these scaffolds in their use for tissue engineering is critically dependent on their chemical, mechanical and surface properties, and microstructure. Here, we present our most recent results on the fabrication of 3D scaffolds from a series of novel materials using Direct fs Laser Writing (DLW) by multiphoton polymerization. These are: (a) Biodegradable polylactide-based scaffolds for tissue engineering applications. We study neuronal and pre-osteoblastic cell growth on them [1]. (b) Organic-inorganic hybrid scaffolds for bone tissue engineering [2]. These are functionalized with amyloid peptides, which selectively immobilize calcium phosphates and hydroxyapatite, generating biomimetic bone scaffolds. We study osteoblast growth to assess their suitability for bone tissue engineering. This study indicates that DLW is a feasible fabrication route for the construction of 3D tissue engineering scaffolds with reproducible feature shape and size.

References:

POL16
Soft-matrices based on fibrous proteins for biofabrication
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Printing various living cells within 3D constructs in defined locations next to each other is a relatively new approach in tissue engineering. Alginate (Alg) is a natural, biocompatible polysaccharide that forms a stable hydrogel. However, it is an inert material and does not promote cell attachment. Herein, our aim is to introduce new hybrid hydrogels to control spatial arrangements of multiple cell types in a defined geometry, by blending alginate with different fibrous proteins, which form homogeneous secondary structures via self-assembly. Thus, in this work the development of novel 2D matrices for further constructions of 3D architectures is presented, aiming to address specific issues in biofabrication. Different fibrous proteins, such as silk fibroin from Bombyx mori, were previously extracted and characterized in terms of protein content (Lowry and SDS-Page). To obtain the hydrogels in 2D and 3D configuration, Alg (2%, w/v) was mixed with fibrous proteins (2%, w/v) to prepare blends of 50/50. To study the swelling ratio and the weight loss, the blends were immersed in HBSS and DMEM, over 21 days, and it was founded that these two parameters are dependent on the composition. The interaction between Alg and different fibrous proteins was further investigated using thermal analysis (DSC) and FTIR assessments. The results demonstrated changes in the protein conformation after blending. Dynamic mechanical thermal analysis (DMTA) on 2D films revealed viscoelastic behavior. Moreover, the cell-material interaction of the hybrid materials was conducted using Human endothelial and fibroblast cells. Together with SEM, viability and live/dead staining measurements showed no cytotoxic effect and the spread of cells in all Alg/fibrous/protein hydrogel. Therefore, it can be concluded that the synergistic effects of each blend component allow the improvement of the properties of the material used to prepare the 2D and 3D geometries. Furthermore, the gel-network and mechanical properties were assessed and tuned by analyzing the response of different cell types to obtain an ideal hydrogel system suitable for biofabrication.
A cell spanning ikvav expressing peptide for treatment of spinal cord injury

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Spinal cord regeneration following local treatment with a membrane spanning peptide (MSP) expressing the IKVAV epitope was assessed following compression injury in Balb-c mice. The day after hemilaminectomy and compression injury, mice were treated with one of the following: isoleucine-lysine-valine-alanine-valine (IKVAV), IKVAV-MSP, peptide and mannitol/saline (vehicle). Functional improvement in movement was assessed daily using Basso Mouse Scale (BMS) and spinal cord segments were studied histologically 28 days after injury. The BMS score for the IKVAV-MSP group increased significantly compared to IKVAV, mannitol and normal groups but not with the MSP-control group (P < 0.05). The number of protoplasmic astrocytes in the IKVAV-MSP mice was significantly increased compared to IKVAV, mannitol and normal groups but not with the MSP-control group (P < 0.001). Neuron and muscle bundle size were also increased significantly (P < 0.05 and P < 0.007, resp.) in the IKVAV-MSP group compared to other treatment groups. The observations in this study demonstrated that it is possible to promote functional recovery after SCI using bioactive IKVAV presenting cell membrane spanning peptides.

High-throughput skeletal stem cell separation using magnetic labelling and microfluidic sorting

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Medical advances have led to a welcome increase in life expectancy. However, this progress presents its own new challenges: increases in age-related diseases, and associated reductions in quality of life, have substantial socio-economic cost. OA is the most common form of arthritis worldwide and the sixth leading cause of disability and in severe cases, necessitating joint replacement using non-biological prostheses. A major limitation in the use of prostheses is the risk of infection, dislocation, mechanical mismatch and functional failure; all leading to revised implants and further surgery. Cell-based therapies are currently some of the most exciting and promising areas for bone disease treatment and reparative medicine. SSCs present in bone marrow (BM) contribute to the regeneration of mesenchymal tissues such as bone, muscle, ligament, tendon and stroma. However, despite intensive research interest, there are currently no reliable methods to isolate (or enrich sufficiently) homogeneous skeletal stem cell populations needed for these strategies given their paucity; less than 0.01%, in bone marrow. This research seeks to develop SSC isolation techniques using unique microfluidic strategies. Traditional immunological sorting methods such as fluorescence/magnetic activated cell sorting (FACS/MACS) can be used to isolate SSCs according to surface marker expression. Both techniques have limitations with regards to purity (~70%), cell viability (20-25% post sorting), running cost, mechanical complexity and the need for trained dedicated technicians, especially FACS. A microfluidic-based approach offers reduced running costs and enhanced homogeneous stem cell and progenitor enrichment. Here, we detail innovative approach to isolate, sort and characterise SSCs from human BM stromal cells (HBMSCs) using a microfluidic device. Functionalised super-paramagnetic beads with adsorbed STRO-1 antibody were used to target the SSC population. Immunomagnetically labelled cells experience a drag force due to a magnetic field generated by thirty permanent neodymium magnets. The system, designed on the same principles as conventional MACS has the added advantage of continuous flow enabling labelled cell separation. Experiments have been performed using polystyrene beads labelled with magnetic nanobeads to simulate the target cells behaviour, while current work is focussed on isolation of STRO-1+ cells from heterogeneous MG63 human osteosarcoma cells. The final phase will be the assessment of STRO-1+ cell separation from whole adult HBMSC populations using this system. This microfluidic approach offers an innovative approach to skeletal stem cell enrichment with significant therapeutic potential therein for cell isolation for skeletal evaluation and application.
**POL19**

**Mouse fetal neural stem cell preparation and brain transplantation using alginate hydrogels**

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The promise of stem cell therapy to achieve tissue regeneration and replace the lost cells is in particular difficult to achieve in the brain. The three dimensional structure of the complicated network of neural cell, where new cells are rarely added in the mammalian brain after birth, represent a major challenge for the incorporation of the transplanted cells. To enhance the abilities of neural stem cells to be incorporated in the brain in particular, the neural stem cells were cultivated using alginate hydrogels. The cells were isolated from the brains of mouse fetuses 14 days old, and cultivated within the alginate beds of standardized 3 mm diameter. Their survival, differentiation and the behavior after the transplantation were followed in different alginate concentrations. The neural stem cells survived and differentiated within alginate beds. The neural markers were present after differentiation. In particular, the alginate concentration suitable for brain transplantation was investigated. The results showed that alginate hydrogels have potential to enhance the abilities of neural stem cells and to serve as a biomaterial supporting their transplantation to the brain. The study was supported by EU FP7 grant GlowBrain (REGPOT-2012-CT2012-316120).

**POL20**

**Peptide-amphiphiles functionalization via co-assembly for cell culture**

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Efforts in biomolecular nanotechnology are increasingly directed towards structural control of supramolecular self-assembly. Developments in this area of research are often inspired by living systems. Peptide-based self-assembly is attracting attention in mimicking intra- and extra-cellular fibrous networks with minimal complexity. It was previously found that using Fmoc-dipeptide gelators creates hydrogel scaffolds that are too hydrophobic for certain types of cells. Co-assembly of Fmoc-dipeptide fibres with a surfactant-like peptide derivative provides a handle for introducing chemical functionality, giving rise to more hydrophilic fibre surfaces that may contain functional peptides into the hydrogels. In this study, we demonstrated a facile supramolecular approach for the formation of functionalized nanofibres by combining the advantages of biocatalytic self-assembly and surfactant/gelator co-assembly. This is achieved by enzymatically triggered reconfiguration of free flowing micellar aggregates of pre-gelator (Fmoc-Fyp) and functional surfactants Fmoc-X (X = S, T or RGD, where RGD is a well-known cell adhesion motif) to form nanofibres that become coated with the surfactants. This results in the formation of fibers that display the functionality at the surface. Furthermore, by varying enzyme concentration, the gel stiffness and supramolecular organization of building blocks can be varied. Next step would be functionalization of peptide fibers with sugar-amphiphiles (e.g. Fmoc-Galactosamine) followed by testing their ability to bind to some carbohydrate-binding proteins (e.g. lectin). These results would give an indication on the ability of these scaffolds to bind to certain types of cells (e.g. hepatocytes).