For instance 30 M presented elasticity higher than the 15 M cells/mL. The obtained data points to the importance of analyzing the reciprocity between cells and matrix for the improvement of cell delivery systems. Acknowledgments: FEDER funds through COMPETE and FCT (project PTDC/SAU-BEB/101235/2008 & FCOMP-01-0124-FEDER-010915).

29.05
Gellan gum-based bilayered scaffolds for application in osteochondral tissue engineering

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Bilayered scaffold and cellular strategies are currently applied to solve the challenging problem of osteochondral defects. In this study, several formulations of Gellan gum were developed to fabricate different scaffolds possessing a cartilage-like layer and a bone-like layer. The bone-like layers were obtained by low acyl Gellan gum (LAGG) at 2 wt% and different amounts of hydroxyapatite powders (HAp) (5,10,15 and 20wt%). The cartilage-like layers were obtained by preparing LAGG formulation at 2wt% and formulations of LAGG at 2wt% and high acyl Gellan gum (HAGG) at 0.75wt% at a ratio of 75:25(v%). The viscoelastic measurements were performed using a TRITEC8000B DMA to characterize the mechanical behaviour of the bilayered scaffolds. The effect of the incorporation of different amount of HAp within the bone-like layer on the mechanical properties of the scaffolds was also investigated. Degradation and water uptake studies were performed by soaking the scaffolds in a phosphate buffered saline solution (pH 7.4) up to 30 days. The bilayered scaffolds were investigated by stereo microscope to evaluate the interface between both layers. The cytotoxicity of the bilayered scaffolds was investigated in vitro using a L929 cell line. In vitro studies regarding adhesion, encapsulation and viability of human chondrocytes (cartilage-like layer) and human osteoblasts (bone-like layer) cultured in the bilayered scaffolds were also carried by performing SEM analysis and Live/Dead assays.

29.P01
Development and optimisation of a growth factor delivery hydrogel for cartilage regeneration

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Engineered hydrogels containing TGF-β loaded microspheres and seeded with mesenchymal stem cells have been under investigation for cartilage repair and regeneration. In this study the influence of crosslinking conditions on TGF-β release from gelatin microspheres was evaluated and two polysaccharide hydrogels (agarose and gellan gum) were compared with fibrin to determine the most suitable hydrogel system for promoting chondrogenesis of progenitor cells. Agarose, fibrin and gellan gum hydrogels were seeded with infrapatellar fat-pad derived stem cells and TGF-β 1 loaded gelatin microspheres. TGF-β 1 release was quantified by ELISA. Constructs were cultured for 21 days and evaluated by biochemical assay and histological staining. TGF-β 1 release was dependent on the crosslinking time used to manufacture the microspheres. After 21 days in culture, sGAG synthesis was higher in both agarose and gellan gum compared to fibrin hydrogels. In addition fibrin underwent considerable contraction after 21 days in culture while agarose and gellan gum maintained their original shape. Histological staining demonstrated the presence of collagen and sGAG in the polysaccharide hydrogels. In conclusion, these results indicate that polysaccharide hydrogels are preferential to fibrin for use as a growth factor delivery scaffold for cartilage repair. The high chondrogenic potential of stem cells cultured in these hydrogels demonstrates the potential of these biomaterials for cartilage regeneration.

29.P02
Chitosan membranes for spatially controlled cell adhesion and specific cell recruitment

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We propose a concept of biomaterials that are able to fix specific cell types onto their surface when in contact with a mix population of cells. Adipose tissue has shown to be an interesting source of stem cells with therapeutic potential. However only a small amount of the heterogeneous mixture of the cells extracted from lipoaspirates are stem cells, and within stem cells there are different populations with different capabilities to differentiate through a lineage. We studied the ability of immobilized antibodies on chitosan surfaces to capture specific types of cells with a spatial micrometer resolution.Antibodies were covalently immobilized onto chitosan membranes using bis[sulfosuccinimidyl] suberate (BS3). X-ray photoelectron spectroscopy (XPS) was used to chemically characterize the surface and quartz crystal microbalance (QCM) to calculate the amount of adsorbed and/or immobilized antibody. Data shown greater immobilization when BS3 was used compared to simple adsorption. Specific antibodies covalently immobilized in a surface, kept their bioactivity and controlled the type of cell that attached on the chitosan surface. Microcontact printing permitted to covalently immobilize antibodies in patterns allowing a spatial control in cell attachment. Cell sorting experiments performed using a mixture of adipose stem cells and osteoblast like cells shown that chitosan surfaces were able to capture a specific phenotype depending on the immobilized antibody.

29.P03
Chitosan-bioactive glass hybrid films and scaffolds: assessment of bioactivity and mechanical behavior

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Many efforts have been made in the development of porous matrices with the objective of increasing its strength for a potential application in bone regeneration. The objective of this study was to investigate a composite material having as reinforcement phase bioactive glass. Hybrid scaffolds and hybrid films were developed with 20% content of bioactive glass (60% SiO2, 36%CaO and 4% P2O5) on chitosan crosslinked with 3% glutaraldehyde, and the introduction of the bioactive phase carried out by the sol-gel method. The films were obtained by solvent evaporation technique and the porous scaffolds were obtained by lyophilization method. The mechanical behavior was investigated by tensile test of the films and compression test of the scaffolds. The hybrid films with 20% bioactive glass showed a significant increase in its tensile strength. The results of in vitro tests with SBF (simulated body fluid) showed the formation of a layer of fibrils on the film surface, upon analysis, that is the characteristic morphology of carbonated hydroxyapatite, reflecting its favorable bioactivity. The porosity and interconnectivity of scaffolds obtained also were confirmed by SEM, which showed a homogeneous structure. The results also showed films with pH-stable after immersion in SBF. The FTIR confirms the presence of characteristic functional groups of the bioactive glass, confirming its introduction into the polymer network of chitosan.