Posters: XXXVIII Annual ESAO & IV Biennial IFAO Congress, 9-12 October 2011, Porto, Portugal

Conclusions: The *in ovo* quantification method was more complex as compared to *ex ovo*. The results indicate that no differences exist between the hydrogels tested in what concerns to their angiogenic potential.

P224 (EI0300)
A GREEN APPROACH TO PROCESS SEMI-CRYSTALLINE NATURAL-BASED POLYMERS FOR TISSUE ENGINEERING APPLICATIONS
A.R.D. Duarte[1], S.S. Silva[1], J.F. Mano[1], R.L. Reis[1]
[1]3B's Research Group - Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Taipas, Guimarães, Portugal

Objectives: Following the green chemistry philosophy, this work aims at designing and developing new 3D architectures of natural-based polymers, combining ionic liquids (ILs) and supercritical fluid technology, with relevant applications in tissue engineering and regenerative medicine. With this purpose, SPCL, a polymeric blend of starch, which is one of the most abundantly occurring natural polymers and poly(ε-caprolactone), a synthetic biodegradable polymer, was processed by supercritical fluid foaming, at different operating conditions. The use of this technique for processing natural-based polymers has been limited due to the fact that they are normally semi-crystalline polymers. This can be overcome by the use of ILs, which have recently been proposed as plasticizing agents of starch.
Methods: The IL tested in this work was 1-butyl-3-methylimidazolium acetate and its plasticizing effect was demonstrated by the mechanical tests conducted. The production of porous and interconnected structures was carried out, hereafter, using CO2 as foaming agent. The effect of different operating variables, such as pressure, temperature and contact time on the porosity, interconnectivity and pore size distribution of the matrixes was evaluated and the morphology was analyzed by micro-computed tomography.
Results and Discussion: The results obtained suggest that the induction of porosity within the constructs depends largely on the diffusion of CO2 in the matrix, which explains the higher porosity of the samples processed at higher pressures and larger contact times. Moreover, the presence of IL has been shown to have a key role in the success of the supercritical foaming process, and consequently on the preparation of porous and interconnected scaffolds.
Conclusions: To our knowledge it is the first time that this approach has been reported. The findings described in this work can be extended and adapted to other raw materials, which largely broads the spectrum of natural-based polymers that may be processed into 3D porous matrixes.

P225 (EI0299)
PLLA-PEG CRYOSTRUCTURED SCAFFOLDS REINFORCED WITH BIODEGRADABLE FIBERS
L. Tutkun[1], S. Egri[2], E. Piskin[1]
[1]Bioengineering Department and Biyomedtek, Hacettepe University, Ankara, Turkey; [2]Bioengineering Department, Gaziosmanpasa University, Tokat, Turkey

Objectives: Poly(l-lactide) (PLLA), poly(glycolide) (PGA), poly(ethylene glycol) (PEG) and/or copolymers of these have extensively been used in medicine for applications. VICRYL® is a commercially available suture produced from a copolymer of glycolide/lactide (90/10 mol/mol). In this study, Vicryl® fibers were used for increasing mechanical properties of PLLA-PEG cryostructures.
Methods: PLLA-PEG block copolymer was synthesized by ring opening polymerization of L-lactide dimer and PEG (6000 Da). Wet spun fibers were obtained from chitosan solution in acetic acid. Commercially available Vicryl® was used as a source of Poly(lactide-co-glycolide) (PLGA) fibers. PLGA fibers with varying length (1, 2 and 4mm) and matrix/fiber ratios (2:1, 3:1 and 4:1) were dispersed in % 5 (w/v) solutions of PLLA-PEG in 1,4 Dioxane. Porous scaffolds were prepared with these solutions under cryotropic conditions (-12°C).
Results: FTIR, and NMR spectra confirmed the chemical structure of PLLA–PEG copolymer. Cryostructures made from this copolymer had interconnected macropores, which were obtained. They exhibited remarkable properties, including high flexibility and rapid size change to external forces, and also "swellability" in aqueous media. Vicryl fibers (both the amount and the fiber aspect ratio) increased the mechanical strength of the PLLA-PEG cryostructures quite significantly.
Conclusions: PLLA-PEG cryostructures reinforced with vicryl fibers were concluded to be a good candidate that can be used in tissue engineering applications for both hard and soft tissue regeneration.

P226 (EI0292)
VALORIZATION OF CHITOSAN FROM SQUID PENS AND FURTHER USE ON THE DEVELOPMENT OF SCAFFOLDS FOR BIOMEDICAL APPLICATIONS
L.L. Reys[1,2], S.S. Silva[1,2], J.M. Oliveira[1,2], A.M. Frias[1,2], J.F. Mano[1,2], T.H. Silva[1,2], R.L. Reis[1,2]*
[1]3B's Research Group - Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Taipas, Guimarães, Portugal; [2]ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal

Objectives: The aim of the present work is the valorization of squid pens through the production of chitosan that can be used for the development of biomedical applications. The present work is focused on β-chitin extraction from squid pens of the species *Dosidicus gigas* and its further conversion into chitosan. The biomedical potential of the isolated squid chitosan was assessed by processing this polymer as scaffolds for tissue engineering strategies.
Methods: Alkali solution was used to deproteinized squid pens and thus isolate β-chitin, which was further converted into chitosan through a deacetylation reaction. The chitosan scaffolds were developed using a freeze-drying process, from 3% and 4% chitosan solutions in acetic acid and freezing at temperatures of -80ºC and -196ºC. Chitosan scaffolds were neutralized using two different methods: M1 – NaHO solution; and M2 – ethanol/water and NaHO solution. Morphology, Mechanical properties, degradation, cytotoxicity (L929 cells) and cellular adhesion (ATDC5 Chondrocytes like cells) of squid chitosan scaffolds were assessed and compared with the properties of scaffolds produced with commercial chitosan.
Results: The morphology of scaffolds revealed a lamellar structure for all produced scaffolds, independent of the origin and concentration of chitosan. The treatment with sodium hydroxide and ethanol caused the formation of larger pores and loose of some lamellar features. Different freezing temperatures gave different pore morphology and the lower temperature a smaller pore size. The *in vitro* cell culture and cell adhesion studies showed that all chitosan scaffolds exhibited a non-cytotoxic effect over the mouse fibroblast-like cell line, L929 cells.
Conclusions: The chitosan produced from the endoskeletons of giant squid *Dosidicus Gigas* has proven to be a valuable alternative to the commercial one when considering its use as biomaterial for different biomedical applications.

P227 (EI0203)
ENCAPSULATION OF HUMAN MESENCHYMAL STEM CELLS VIA PROTEIN CROSS-LINKING FOR INTERVERTEBRAL DISC REGENERATION
A. Schmiermund[1], D. Freimark[1], H.-L. Fuchsbauer[2], P. Czermak[1,3]
[1]Inst. of Bioprocess Engineering and Pharmaceutical Technology, University of Applied Sciences, Mittelhessen, Germany; [2]Dept. of Chemical and Biological Engineering, University of Applied Sciences, Darmstadt, Germany; [3]Dept. of Chemical Engineering, Kansas State University, Manhattan KS, USA

Objectives: Low back pain is a common disease in modern society. One major reason is the degeneration of the nucleus pulposus (NP, part of the intervertebral disc). The regeneration of nucleus pulposus via cell therapy requires a biocompatible matrix, which facilitates the differentiation of hMSCs (human mesenchymal stem cells) to nucleus pulposus cells. The enzyme transglutaminase was found to be suitable to generate a gelatine matrix by cross-linking and could be utilized to immobilize hMSCs. Further natural ECM of the NP should be used for crosslinking and immobilization.
Methods: The immobilized cells were cultivated for 21 days in media, containing growth and differentiation factors. Cells were seeded at a density of $4x10^6$ cells per cm^3, equal to the density in the NP. Viability of the cells in the gelatine matrix was proofed by using a tetrazolium salt (WST-1). At certain dates RNA isolation was done following the phenol/guanidine isothiocyanate protocol. RNA was transformed into cDNA followed by a RT-PCR with a gel electrophoresis afterwards. Different primers were used to analyze the successful differentiation of hMSC-TERT into nucleus pulposus cells. In further analysis, NP extract isolated from pigs, has been added to the gelatine matrix.
Results: The viability of the immobilized cells has been on a constant value over the differentiation period of 21 days. Thus, the survival of hMSC-TERT in gelatine is proofed. The differentiation status of hMSC-TERT in the two different matrices (gelatine, NP extract) could be analyzed.
Conclusions: Data concerning the differentiation of hMSC-TERT in nucleus pulposus cells in a gelatin matrix and differences between the two matrices will be presented.