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

into organ hemodynamics, perfusion and (dys)function (e.g. transplant research). Previously, we developed an electrical analog model of the human hepatic circulation, based on measured anatomical data of the macrocirculation. However, this model requires refinement, especially at the microcirculatory level.
Methods: Vascular corrosion casts of two human livers (discarded for transplantation) were obtained by simultaneous injections of Batson's™#17 through the hepatic artery (HA) and portal vein (PV). We previously reported data obtained from an in globo micro-CT scan (resolution ±110µm) of one of the liver casts. As this only allowed assessing 5-6 generations, we dissected a lobe and a small sample (±0.134mm³) from the cast and scanned these at higher resolutions (±71µm and ±2.6µm, respectively). Image processing was performed to obtain 3D reconstructions up to the terminal microcirculatory level. These reconstructions enabled measurements of the branching topology (diameters, radii, lengths), and assessment of the microvascular porosity (void volume divided by total volume).
Results: The dissected lobe dataset resulted in the visualization of higher order blood vessel generations [13 for the HA and PV, 10 for the hepatic veins (HV)]. Exponential relations [$y=a\exp(bN)$ with N=generation number] were determined based on data from the 1st to 13th/10th generation, relating generation number to radius, length and number of vessels. For the HA/PV/HV radii [mm], *a* was 4.22/10.26/13.52, while *b* was -0.31/-0.37/-0.48, respectively. The smallest sample showed a very complex network of interconnected and intertwined sinusoids (diameters of ±5-10µm), and the porosity was estimated at 0.148±0.007.
Conclusions: We gathered anatomical and morphological data on the hepatic macro- and microcirculation, forming the basis of a multiscale model of the liver blood flow and perfusion. The application of this method could also be extended to other organs, such as kidneys.

Natural-based polymeric biomaterials and composites for regenerative medicine

P221 (EI0369)
NOVEL GELLAN GUM HYDROGELS FOR TISSUE ENGINEERING OF INTERVERTEBRAL DISC
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Objectives: Centrally situated in the intervertebral disc (IVD) structure, the nucleus pulposus (NP) is a gel-like tissue containing proteoglycan (PG) such as versican, biglycan, decorin, fibromodulin, luminican and especially aggrecan. NP has an important structural role in the IVD, and upon damage it possesses a limited self-repair capacity. Therefore, the main purpose of this work was to develop novel gellan gum-based hydrogel (GG) formulations consisting of GG MPs dispersed in a GG matrix for finding application as an NP substitute.
Methods: Several GG MP/hydrogels discs formulations were prepared by means of mixing high and low acyl GG at different ratio, namely 75%:25% (v/v); 50%:50% (v/v), 25%:75% (v/v); HAGG 0.75% and LAGG 2%, respectively. The GG MP/hydrogel discs formulations were investigated by dynamic mechanical analysis (DMA), swelling behavior and degradation rate. The possible cytotoxicity of MP/hydrogel discs leachables was screened *in vitro* by means of using a rat lung fibroblast-like cell (L929 cells) line. In order to qualitatively investigate the encapsulation efficacy of L929 cells into the GG MP/hydrogel discs a Live/Dead cell viability assay was also carried out.
Results and Discussion: The developed GG MPs/hydrogel discs were physico-chemically characterized by FTIR and ^{1}H-NMR, and GG MPs size was measured by a stereomicroscope by means of staining the MPs with Toluidine Blue-O. From DMA analysis, we observed that the optimal outcome to reinforce GG matrices may be in the range of 50-500 mg/mL of incorporated MPs. The cell culture studies demonstrated that MP/hydrogels discs are non-cytotoxic over L929 cells. Complementarily, it was also demonstrated that L929 cells can be successfully encapsulated in the GG MP of different formulation and that were viable after 72 hours of culturing.
Conclusions: The developed GG MPs/hydrogel discs are promising hydrogels for being used in IVD tissue engineering applications.

P222 (EI0323)
SUBCRITICAL SINTERING OF AN ALIPHATIC POLYESTER ENRICHED WITH ULVAN CAPSULES LOADED WITH A BIOACTIVE AGENT
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Objectives: A marine derived polysaccharide, ulvan, extracted from green algae, was combined with Poly-DL-Lactic acid (PDLLA) in order to produce a novel scaffold targeted for Tissue Engineering (TE) applications. The combination of this natural origin polysaccharide with PDLLA aimed at the improvement of the scaffold performance and physical integrity while broadening drug release capabilities.
Methods: 3D scaffolds of PDLLA loaded with ulvan beads were prepared by subcritical fluid sintering with carbon dioxide at 40°C and 50 bar. The prepared matrices were further characterized by several techniques as to assess their feasibility as scaffolds for bone regeneration, namely mechanical compression testing, water uptake and degradation studies, morphological characterization by micro-computed tomography (µ–CT) and scanning electron microscopy (SEM) and standard biocompatibility tests (ISO/EN 10993). Effectiveness of ulvan particle loading with dexamethasone within the PDLLA matrix was monitored in terms of release profile of the compound by UV spectroscopy.
Results and Discussion: PDLLA/ulvan 3D structures exhibited a compressive modulus of 8.3±1.51 MPa, a maximum water uptake of 8% and weight loss of 5%, after 21 days. In terms of morphometric properties, these scaffolds presented a porosity of 75.7±1.24%, pore interconnectivity 48.7±8.49% and a mean pore diameter of 268.5±12.02µm. The release of dexamethasone from the ulvan particles demonstrate that the systems designed can be successfully used for *in situ* delivery of bioactive agents.
Conclusions: The obtained results demonstrated the feasibility of processing PDLLA/ulvan 3D scaffolds for potential TE scaffolds. These structures presented appropriate mechanical and morphological characteristics to be used as a scaffold for cancellous bone tissue engineering. They also contributed to the establishment of ulvan as an emerging biomaterial candidate for TE applications.

P223 (EI0394)
***IN VIVO* STUDY ON THE ANGIOGENIC POTENTIAL OF GELLAN GUM-BASED HYDROGELS FOR APPLICATION IN NUCLEUS PULPOSUS REGENERATION**
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Objectives: Tissue engineering of nucleus pulposus (NP) offers a promising alternative strategy to current ineffective clinical approaches for treating intervertebral disc degeneration. Gellan gum-based hydrogels (ionic- and photo-crosslinked methacrylated gellan gum) have been recently proposed as potential candidates for NP regeneration. An important feature of these hydrogels will be their capacity to control blood vessel growth, since the NP is naturally avascular. Our aim was to investigate *in vivo* the angiogenic/antiangiogenic potential of the developed hydrogels, using an optimized adaptation of the chorioallantoic membrane (CAM) assay.
Methods: Sterile hydrogel discs (n=10) made of gellan gum, ionic- and photo-crosslinked methacrylated gellan gum were placed on the CAM at day 10 of embryonic development. Positive (filter paper or gelatin sponge with VEGF) and negative (filter paper or gelatin sponge) controls were also tested. The assay proceeded until day 14 or 18 of embryonic development and images were acquired *in ovo* and *ex ovo* using a stereomicroscope by the end of the assay. The images obtained were image-processed using the ImageJ program for facilitating the counting, which was performed by three independent observers.
Results: The evaluation of the angiogenic response was performed by analysing the convergence of the blood vessels toward the implanted discs. Some degree of variability was found between replicates and inflammation occurred frequently, which hindered the analysis of the formation of new blood vessels. The reduction of the assay duration (from 18 to 14 days) resulted in a decrease of inflammation/contamination. All the materials were partially adsorbed during the assay. However, the controls did not present a regular response and the gelatin sponge was often completely adsorbed.