

**(P 298) Optimization of Chitosan-Based Composite and Bilayered Scaffolds Produced by Particles Aggregation for Osteochondral Tissue Engineering: Influence of Hydroxylapatite**

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Osteochondral tissue engineering presents a challenge to the present research due to requirements' combination of both bone and cartilage tissue engineering. In the present study, bilayered chitosan scaffolds are proposed based in the optimization of polymeric and composite scaffolds.  $\mu$ -CT was carried out for accurate morphometric characterization quantifying porosity, interconnectivity, ceramic content, particles and pores size. The results showed that the developed scaffolds are highly interconnected and present ideal pore size range, being morphometrically adequate for the proposed applications. DMA shown that scaffolds are mechanically stable in wet state under dynamic compression solicitation. The obtained elastic modulus at 1 Hz frequency was  $4.21 \pm 1.04$  MPa,  $7.98 \pm 1.77$  MPa and  $6.26 \pm 1.04$  MPa for polymeric, composite and bilayered scaffolds, respectively. In composite scaffolds, surprisingly it was observed a cytotoxicity behaviour when unsintered hydroxylapatite was used. This study reports the investigation that was conducted to overcome and explain this behaviour. Cytotoxicity was evaluated by MTS with L929 fibroblast cell line for different conditions and ICP was carried out to confirm the influence of several elements. Sintered hydroxylapatite was further used showing no cytotoxicity. Bioactivity studies with simulated body fluid (SBF) and simulated synovial fluid (SSF) were conducted to assure that the polymeric component for chondrogenic part would not mineralized as confirmed by SEM, ICP and EDS for different immersion periods. It is concluded that chitosan-based bilayered scaffolds produced by particle aggregation could serve as alternative, biocompatible, and safe biodegradable scaffolds for osteochondral tissue engineering applications.

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