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INTRODUCTION: A lot of effort has focused on the development of bone graft substitutes to accelerate bone repair. Silk fibroin scaffolds (SFSC) have shown to be highly suitable for bone tissue engineering (BTE) [1]. SFSC can be produced by several methods, such as salt leaching (SL) or inverse opal (IO). Compared to salt leached scaffolds (SLSC), IO scaffolds (IOSC) are homogeneous and structured scaffolds. IOSC have been shown to enhance extracellular matrix (ECM) mineralization compared to SLSC under static cell culture conditions, suggesting their potential for BTE. Differences in mineralized ECM were attributed to pore diameter distributions or pore geometry. BTE constructs aim to mimic the structural but also the mechanical characteristics of natural tissue [2]. It is well known that cells respond to mechanical stimuli. Incorporation of bioreactors in BTE eliminates nutrient transfer limitations of static cultures but also provide mechanical stimulation to the cells, mimicking the *in vivo* environment of bone. The aim of this study was to assess the potential of IOSC for BTE applications. In this study, we evaluated and compared the osteogenic effects of SFSC with two pore geometries SL and IO, respectively - under mechanical stimulation. Molecular biology and imaging techniques were combined to provide a better insight on how pore geometry influences how cells respond to mechanical stimuli. A better understanding on how cells sense and respond to mechanical stimulation depending on the scaffold pore geometry will help on the design scaffolds for BTE.

METHODS: SLSC were prepared as described previously [3]. IOSC were prepared by assembling monodisperse and spherical pore template microparticles into a crystalline lattice before infiltration with aqueous silk fibroin solution. Human mesenchymal stem cells were cultured on both scaffold types for up to 49 days with osteogenic supplementation in spinner flask bioreactors (70rpm). Cell distribution and collagen synthesis was assessed by Haematoxylin and Sirius red (SR) staining. Expression of bone related genes was quantified by real-time RT-PCR. Formation and spatial distribution of mineralized ECM was evaluated by micro-computed tomography.

RESULTS: Although spinner flask bioreactors supported homogeneous mineralized ECM formation in both IOSC and SLSC, the amount of mineralized ECM volume fraction (BV/TV) was nearly doubled for IOSC. On the contrary, SLSC enhanced collagen formation, as revealed by SR staining (especially at the edge of the scaffolds) and significantly upregulated of Collagen-I (Coll-I) ($p=0.016$) and Osteocalcin (OC) gene expression ($p=0.001$) compared to IOSC.

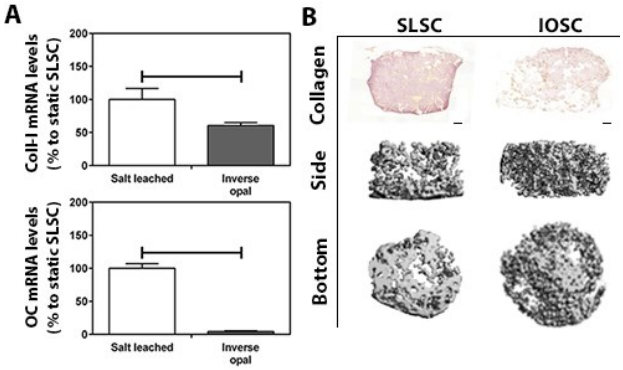

Fig. 1: (A) Coll-1 and OC relative mRNA levels, (B) collagen formation (scale bar 500µm) and 3D mineralized ECM.

DISCUSSION & CONCLUSIONS: Based on the results observed, it can be assumed that the differences in pore geometry of IOSC compared to SLSC have a direct effect on how cells sense and respond to mechanical stimulation. These results might suggest that homogeneous and structured IOSC enhanced cell differentiation and thus formation of a highly mineralized tissue compared to SLSC, however further research will be needed.

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