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JR Vetsch[1], R Müller[1], S Hofmann[1,2,3]

[1] *Institute for Biomechanics, ETH Zurich, Zurich, CH.* [2] *Department of Biomedical Engineering and* [3] *Institute for Complex Molecular Systems, TU Eindhoven, Eindhoven, NL.*

INTRODUCTION: The adult skeleton constantly adapts its structure to changes in long-term mechanical loading. This continuous process of bone resorption and formation is called bone-remodelling [l]. The refilling of resorption sites *in vivo* has been shown to be most probably curvature driven [2]. *In vitro*, essential work on the influence of curvature on non-mineralized tissue in static cultures has been performed. Local curvature increased tissue growth formed by osteoblasts [3]. Cytoskeletal changes have been observed when comparing endothelial cells cultured on curved or flat surfaces. The changes were additionally enhanced when cells were exposed to perfusion [4].
The effects of curvature and perfusion on mineralized extracellular matrix (ECM) formation have, however, never been observed in 3D. Hence, the aim of this study was to investigate the effects of curvature on different ECM components in a 3D tissue engineered bone construct under static or perfused conditions.

METHODS: Porous silk fibroin scaffolds were produced by introducing three channels of different sizes using biopsy punches. The relative curvatures of the three channels were set to -50% in curvature (S channel, d=1mm) or +50% in curvature (L channel, d=3mm) with respect to the M channel (d=1.5mm). Scaffolds were seeded with $5x10^6$ human mesenchymal stem cells each, supplied with osteogenic medium and cultured in either static or perfused bioreactors (N=10 per group). Micro-computed tomography (µCT) was performed weekly to monitor mineralized ECM formation. Mineralized ECM was evaluated in three different volumes: (i) the 'full scaffold volume', (ii) the 'void volume' of the channel (90% of channel diameter), and (iii) the 'full volume' of the channel (channel diameter plus 1mm). Histology was performed to visualize cell nuclei and non-mineralized ECM stained with Haematoxylin&Eosin (H&E) and collagen stained with Sirius Red (SR).

RESULTS: µCT monitoring showed curvature dependent ingrowth of mineralized ECM into the 'void volume' of the channels (Fig. 1). The coverage of the channels increased with curvature and was additionally increased by perfusion (Fig. 1A-C, right). The morphology of mineralized ECM was more cortical-like in static samples (Fig. 1A-C, left) compared to the more trabecular-like structure in perfused samples (Fig. 1A-C, right).

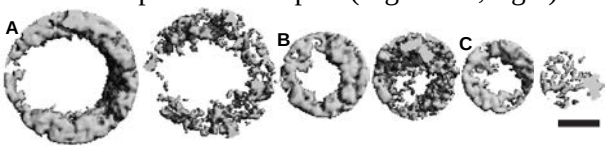

Fig. 1: 3D mineralized ECM in (A) L, (B) M, and (C) S channels (top view). A-C left: static samples. A-C right: perfused samples. Scale bar: 1mm.

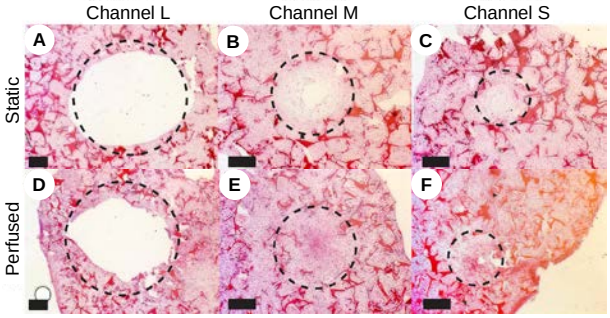

Fig. 2: H&E staining of: (A-C) static and (D-F) perfused samples. Scale bar: 1mm.

Histology images confirmed the results observed by µCT. H&E staining showed less coverage of channels with cells and non-mineralized ECM when cultured under static (Fig. 2A-C) compared to perfused (Fig. 2D-F) conditions. SR staining showed the same patterns for collagen.

DISCUSSION & CONCLUSIONS: This study showed a clear dependence of different ECM components like mineralization or collagen on local curvature. Perfusion showed to enhance the effects of curvature. Additionally, the results observed suggest a possible application of the scaffold model to investigate circular critical size defects in trabecular or cortical bone *in vitro.*

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