Design and Characterization of 3D-Printed Hydroxyapatite Scaffolds using Synchrotron-Radiation-based Micro Computed Tomography

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INTRODUCTION: To reconstruct the bony tissue within large defects, highly porous biocompatible scaffolds based on hydroxyapatite granulates can serve as 3D templates for initial cell attachment and subsequent tissue formation. The design of the scaffolds has to fulfill different criteria to ensure cell viability and function. These include nanoporosity to allow diffusion of molecules for nutrition and signaling, micropores to ensure cell migration and capillary formation as well as macropores for arteries and veins. Synchrotron-radiation-based micro computed tomography (SRμCT) is a unique, non-destructive technique to characterize the scaffolds with respect to the integral nanoporosity and the detailed morphology of micro- and macropores under in-vitro conditions. Comparing different scaffold designs seeded with progenitor cells, appropriate preparation procedures for the cell-scaffold composites can be uncovered.

METHODS: Three different scaffold structures of cylindrical shape (D = 4 mm, H = 6 mm), shown in Fig. 1, were realized using 3D printing [1]. Multipotent ectomesenchymal progenitor cells isolated from human tooth were cultured in DMEM containing 10% FCS. Subsequently, each scaffold was statically loaded with 80 µl cell suspension containing 2×10^5 cells and incubated for 2 h at 37 °C. The cell-scaffold constructs were harvested after 28 days and fixed in 4% paraformaldehyde for 24 h. The SRμCT measurements were carried out in absorption contrast mode at the beamline BW 2 (HASYLAB at DESY, Hamburg, Germany) using the photon energy of 24.5 keV. The pixel length corresponds to 3.9 µm and the spatial resolution determined by the modulation transfer function to 6.5 µm [2].

RESULTS: Similar to the *compacta*, the 3 designs exhibit a denser outer structure to provide the mechanical stability. As demonstrated in Figs. 1 and 2, a central channel provides space for larger blood vessels and medium flow. Perpendicular to this main channel, 50-100 channels with diameters between 300 and 400 µm allow cell migration. The rounded structures offer a relatively large surface for cell attachment. Finally, the designs ensure that the total X-ray absorption is comparable in all directions perpendicular to the main channel axis.

The SRμCT reveals that the scaffolds are built out of nanoporous granulates forming interconnected microchannels.

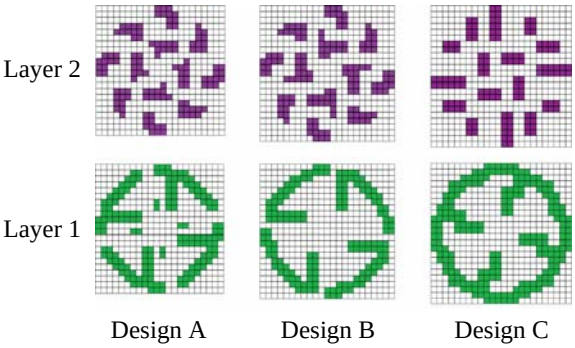

Fig. 1: The scaffolds are formed by alternating layers. One pixel corresponds to 240 µm.

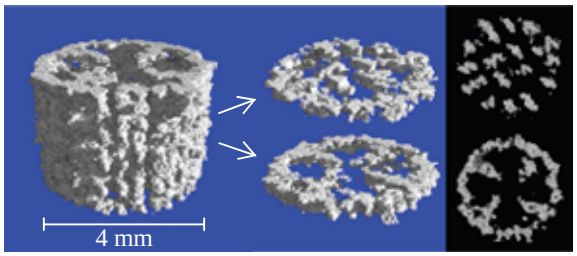

Fig. 2: 3D representation of SRμCT tomogram of design C. Extracted slices with a height of 240 µm are given on the right.

DISCUSSION & CONCLUSIONS: SRμCT allows non-destructively analyzing the porosity of ceramic scaffolds from millimeter to nanometer scale. Therefore, the method provides complementary information to classical histology, avoiding any kinds of preparation artifacts due to sectioning. Due to mechanical stability and the larger number of channels, design C is favored.

REFERENCES: [1] H. Seitz, W. Rieder, S. Irsen, B. Leukers, C. Tille (2005) J Biomed Mater Res Part B: Appl Biomater **74B**:782–88. [2] B. Müller, P. Thurner, F. Beckmann, T. Weitkamp, C. Rau, R. Bernhardt, E. Karamuk, L. Eckert, J. Brandt, S. Buchloh, E. Wintermantel, D. Schwarnweber, H. Worch (2001) Proc. SPIE **4503**:178-88. **ACKNOWLEDGEMENTS:** HASYLAB at DESY Hamburg, Germany (Proposal I-05-028).