13th Swiss Conference on Biomaterials, May 9th 2007, Neuchâtel, Switzerland

Interconnected Porous Scaffolds for Bone Augmentation

Fabienne Fierz[1], Felix Beckmann[2], Barbara Leukers[3], Stephan Irsen[3], Özer Degistirici[3], Michael Thie[3], Marius Huser[4], Adrian Andronache[4], and Bert Müller[1]

[1]*Biomaterials Science Center, University of Basel, Switzerland,* [2]*GKSS-Research Center Geesthacht, Germany,*
[3]*Caesar Research Center, Bonn, Germany,* [4]*Computer Vision Laboratory, ETH Zürich, Switzerland,*

INTRODUCTION

To reconstruct 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.

Tomograms obtained by synchrotron radiation-based micro computed tomography (SRμCT) are the basis for the 3D characterization of the scaffolds on the micrometer scale using sophisticated tools such as distance mapping, component labeling, dilatation/erosion, radial density distributions, and image registration.

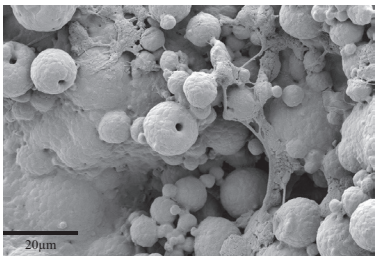
MATERIALS & METHODS

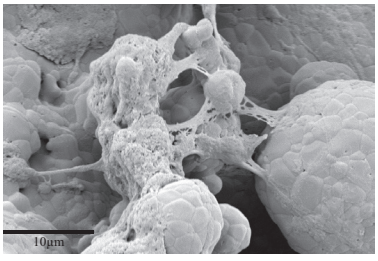
Three open porous scaffolds (design A, B and C) of cylindrical shape (D = 4 mm, H = 6 mm) were fabricated by 3D printing using the pixel size of 240 μm. Similar to the compacta, they exhibit a denser outer structure to provide the mechanical stability. A central channel provides space for larger blood vessels, nerves, or medium flow. Starting from this main channel, 48-72 micro-channels several hundred micrometers thick form an interconnected network.

As building material, spray-dried hydroxyapatite granulates were used. Each scaffold was statically loaded with 80 μl cell suspension containing 200'000 cells isolated from human tooth. The cell-scaffold constructs were harvested after 28 days.

SRμCT measurements were carried out in absorption contrast mode at the beamline W2 (HASYLAB at DESY, Hamburg, Germany) using the photon energy of 30 keV. The pixel length corresponds to 3.7 μm and the spatial resolution determined by the modulation transfer function to 7.4 μm [B. Müller et al. (2001) Proc. SPIE 4503:178-88].

CELL ATTACHMENT

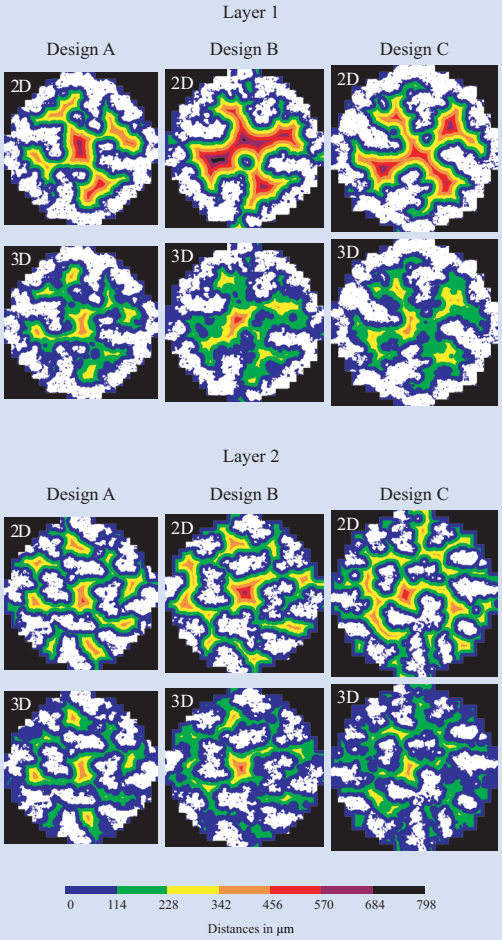




The SEM images uncover the micro-structure of the scaffold and the cell attachment. The cells are located at the surface of the granulates. Only a few cells span over the free space inbetween. In addition, the images demonstrate the crystalline structure of the granulates, showing the grain boundaries as the result of the high-temperature sinter process. Note, that the granulates exhibit a central opening and a nanoporous wall.

The SEM measurements were performed according to standard procedure (5 nm gold) using ZEISS Supra55 at 5 kV accelaration voltage.

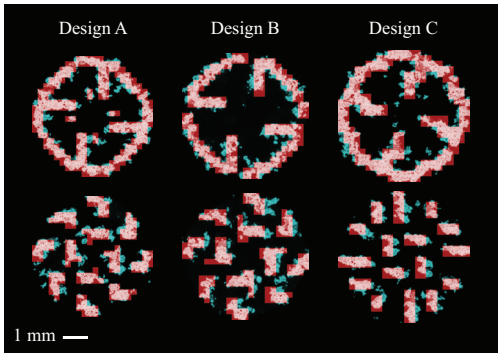
DISTANCE MAPPING



Mean distance to material	Design A	Design B	Design C
2D analysis	133 μm	148 μm	143 μm
3D analysis	100 μm	98 μm	98 μm

The distance map is used to quantify the pore architecture. Here, the minimal distance of each pixel (2D analysis) or voxel (3D analysis) to material is measured and represented by the related color. 2D analysis only gives an upper limit, because material can be present above and below the layer. Consequently, the mean value for the distance transform is larger for the 2D than for the 3D analysis.

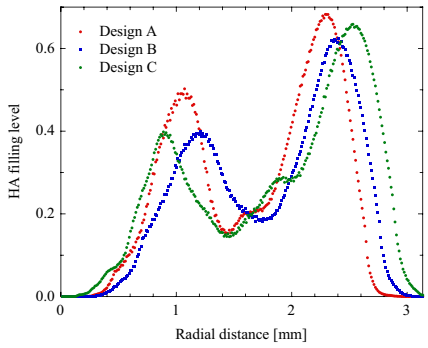
REGISTRATION



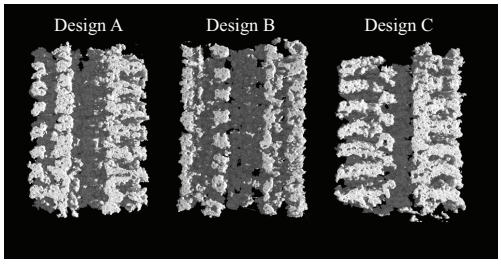
Two selected slices representing layers 1 and 2 of each design are shown. Image registration has been performed to calculate the exact shrinking parameter of the sintered scaffolds. The shrinking is almost isotropic and corresponds to (73 ± 2)%.

The mask is red+white-colored, material outside mask blue and material inside mask white. Relative to the mask, (31±2)% are red-colored and (27±3)% blue, independent on design.

RADIAL DENSITY



The radial density distribution does not only show the total scaffold diameter and the distribution of the building blocks, but also demonstrates that the central channel is up to 1 mm wide (see virtually cut 3D representation of the tomograms below).



CONCLUSION & ACKNOWLEDGEMENT

The digital, 3D data nondestructively obtained by SRμCT are the perfect basis for the visualization and quantification of the scaffold morphology. The application of standard software, however, is difficult because of the huge data size in the range of GB and hence specific code was developed. The distance mapping clearly demonstrates that the 2D analysis overestimates the mean distance to the material by 30 to 50%. The perfect connectivity of the micro-channel network is verified by component labeling. The major air component covers (99.53 ± 0.09)%. By the definition of a smallest diameter that cells can pass, we are able to determine the parts of the cavity accessible for the cells by migration. 3D registration algorithms are shown to allow for the precise measurement of the shrinking as the result of the sintering process and for the detailed evaluation of the scaffolds morphology with respect to the planned design. The quality of the scaffold can be also characterized by means of the radial density distribution. For example, it can support the measurement of the central channel's diameter.

The authors thank HASYLAB at DESY Hamburg, Germany, for beamtime allocation (I-05-028 and II-20060035 EC).