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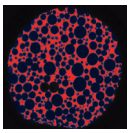
Interconnectivity of Scaffolds for Tissue Engineering

Sarper Gürel[1], Alessandra Braccini[2], Ivan Martin[2], Felix Beckmann[3], and Bert Müller[1]

[1]*Biomaterials Science Center, University of Basel, Switzerland*, [2]*Departments of Surgery and of Research, University Hospital Basel, Switzerland,* [2]*GKSS-Research Center Geesthacht, Germany*

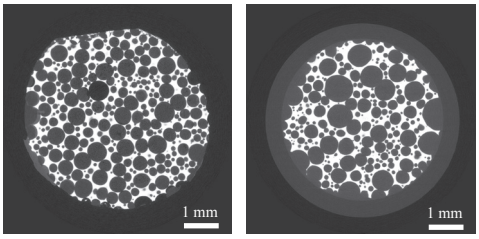
INTRODUCTION



Bone tissue engineering aims to fulfil the need to provide bony tissue for skeletal use. In spite of the fact that the allogenic bone or autogenous grafts have been used for decades, disadvantages like failure of complete resorption of autogenous bone raises the demand to have alternative approaches, which puts bone tissue engineering into play. Usage of three-dimensional (3D) porous ceramic scaffolds in bone tissue engineering manifests itself as a promising methodology for treatment of a wide range of clinical situations, challenging to replace former methods like allografts, synthetic materials etc. Scaffold characteristics such as porosity, interconnectivity and especially morphology on the micrometer scale are crucial for optimizing cell attachment and the related osteointegration.

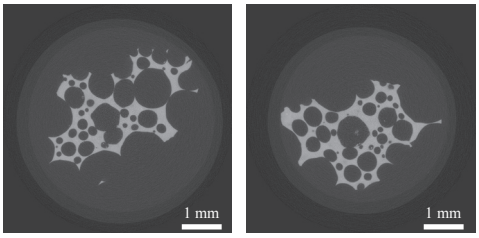
SCAFFOLD ANALYSIS

In this study, the micro-architecture of hydroxyapatite scaffolds with the diameter of 8 mm and with the height of 4 mm is non-destructively uncovered by micro computed tomography. The scaffolds (Engipore; Fin-Ceramica Faenza, Faenza, Italy - www.finceramica.it) have a total porosity of 83% ± 3%. Their pore size distribution can be described as the following: 22% are smaller than 100 µm; 32% between 100 and 200 µm; 40% between 200 and 500 µm and 6% larger than 500 µm. Therefore, these scaffolds provide a micro-architecture that is appropriate for seeding of autogenous bone marrow stromal cells (BMSC).

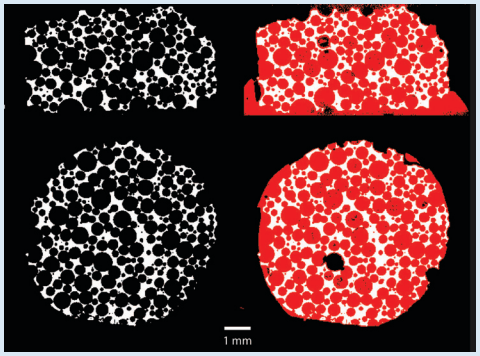


The opaque constructs are made visible by the use of synchrotron radiation-based micro computed tomography (SRµCT) in absorption contrast mode [F. Beckmann et al Proc SPIE 631810 (2006)]. These measurements were carried out at the beamline W2 at HASYLAB/DESY (operated by GKSS-Research Center Geesthacht) using the photon energy of 30 keV. The pixel length corresponds to 4.3 µm and the measured spatial resolution to 7.4 µm [B. Müller et al Proc SPIE 4503 (2002) 178-188]. The data were reconstructed by the filtered back projection algorithm taking into account 721 projections. In the figure above two reconstructed slices were shown. On the left, the scaffold is embedded into paraffin and, on the right, the sample is hold in an Eppendorf container filled with liquid.

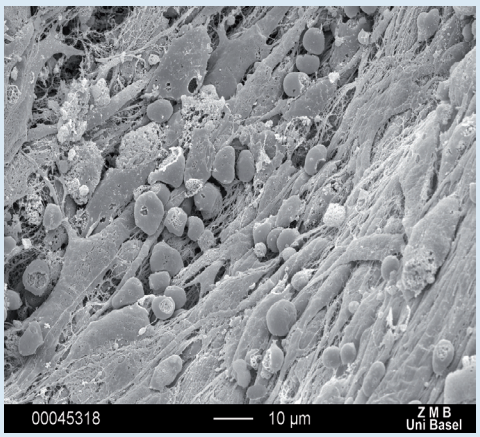
The figure below represents reconstructed slices of a scaffold measured in an Eppendorf container filled with phosphate buffer saline (PBS). The scaffold was seeded with osteoblasts from sheep. The pixel length, here, corresponds to 3.73 µm.



VISUALIZATION



The figure above shows the morphology of the porous scaffold by means of two virtual slices perpendicular to each another. The pores do have a spherical shape. Many of them seem to be connected. In order to determine the degree of interconnectivity the pores were filled with paraffin (red-colored).The paraffin was segmented by intensity-based thresholding possible as the consequence of the lower X-ray absorption with respect to the scaffold material. The paraffin clearly penetrated through all pores. Some pores, however, are incompletely filled. They are just covered at their periphery by a film at least 40 µm thick.



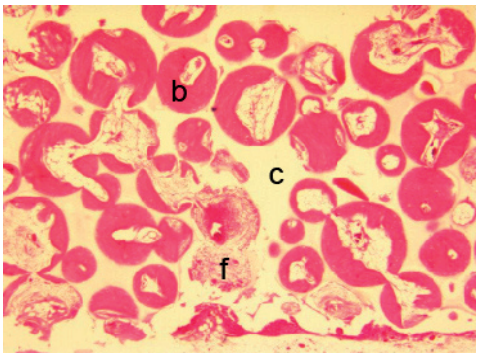
Scanning electron microscopy image of a pore of the generated construct following in vitro culture. The ceramic pore is filled with a stromal-like tissue, consisting of a 3D-network of heterogeneously shaped cells and extracellular matrix.

TISSUE ENGINEERING

Bone tissue engineering based on 3D porous ceramic scaffolds and autologous bone marrow stromal cells (BMSC) is an emerging and promising approach for treating numerous clinical cases, as an advantageous alternative to the currently used clinical methods (e.g. autografts, allografts, synthetic materials). It has been demonstrated that by using a previously developed perfusion-bioreactor system [Wendt et al Biotechnol Bioeng 84 (2003) 205], nucleated cells freshly isolated from human bone marrow aspirates were seeded on hydroxyapatite disks. The obtained cell-scaffold constructs can be further cultured under perfusion, thus generating constructs that, following ectopic implantation in nude mice, form uniform and extensive bone tissue (s. figure in the middle collumn, bottom) [Braccini et al Stem Cells 23 (2005) 1066].

Qualitative characterization of the in vitro generated constructs by scanning electron microscopy indicated the in vitro formation of a stromal-like tissue within the ceramic pores, consisting of a 3D network of spheroidal and fibroblastic cells (figure below) [Braccini et al Stem Cells. 23 (2005) 1066].

With the ultimate goal of 'optimizing' the generation of osteoinductive constructs, further studies are needed in order (i) to characterize the in vitro generated constructs, both in terms of quality and quantity of tissue formed in the scaffold pores during the in vitro 3D-culture, and (ii) to study the scaffold properties (e.g. porosity, pore interconnectivity and diameter, mechanical properties, material composition and entire 3D micro architecture) and the cell-scaffold interactions during the in vitro 3D-culturing and stimulation.



Haematoxylin/Eosin cross-section of the generated construct following ectopic implantation in nude mice. White spaces correspond to the decalcified ceramic (c), whereas scaffold pores are filled with bone (b) or fibrous (f) tissue.

CONCLUSION AND ACKNOWLEDGEMENT

3D micro architecture of porous ceramic scaffolds is made visible using SRµCT. High resolution of imaging in SRµCT in absorption contrast mode permits the detailed visualization of cellular structures and micro-architecture of biomaterials. The visualization of penetrated paraffin revealed that the pores are well interconnected. The channels between the spherically shaped pores, however, are quite thin and are, therefore, sometimes only incompletely filled. The data allows further quantification based on sophisticated computer vision tools including component labelling, growing region and dilatation procedures.

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