Interconnectivity of Scaffolds for Tissue Engineering

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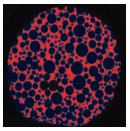
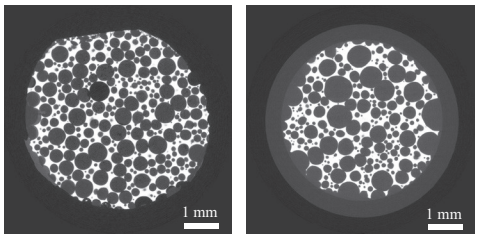
INTRODUCTION



Bone tissue engineering aims to fullfill 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 mechanical properties are crucial for optimizing the osteoinductivity of the generated constructs. Interconnectivity is a prerequisite for mechanical stimulation of the seeded cells.

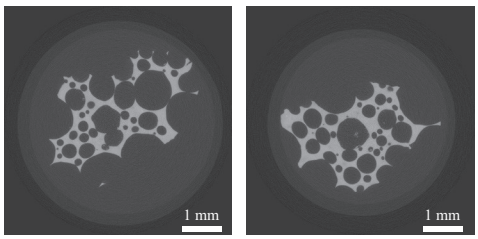
SCAFFOLD ANALYSIS

In this study, the micro-architecture of cylindrically shaped hydroxyapatite scaffolds with the diameter of 8 mm and the height of 4 mm is characterized. The scaffolds (Engipore; Fin-Ceramica Faenza, Faenza, Italy) 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 [1]. Qualitatively, this can be seen in below. Therefore, these scaffolds are appropriate for seeding of autogenous bone marrow stromal cells (BMSC) on scaffold.

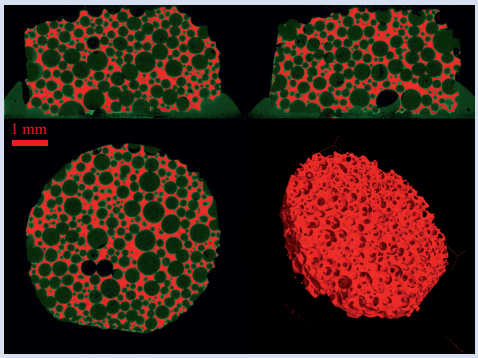


The opaque constructs are made visible by the use of synchrotron radiation-based micro computed tomography (SRµCT) in absorption contrast mode. These measurements with a duration of about 2 hours were carried out at the beamline HARWI-II at HASYLAB/DESY (oerated by GKSS-Research Center Geesthacht) using the photon energy of 30 keV. The pixel length corresponded to 4.3 µm resulting in a measured spatial resolution of 7.4 µm. The data was 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 was embedded into paraffin and on the right the sample was introduced into an Eppendorf container filled with liquid.

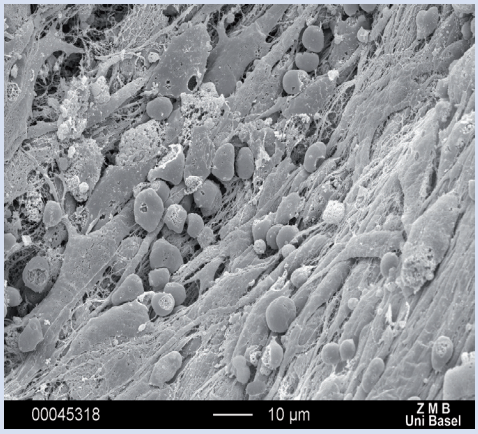
The figure below represents reconstructed slices of a scaffold measured in an Eppendorf container filled with PBS. The scaffold was seeded with osteoblasts from sheep. The pixel length correspond to 3.73 µm.



VISUALIZATION



The 3D representation of hydroxyapatite scaffold (red colored) shows the interconnected spherical pores together with frontal, sagital and axial cuts, respectively. The interconnectivity is demonstrated by the green color representing paraffin, which was used as embedding material. Only a few pores are partially filled or not penetrated (see dark areas inside). It is planned to quantify the interconnecting canals of the scaffolds by means of sophisticated computer vision tools including component labeling, growing region and dilatation procedures.



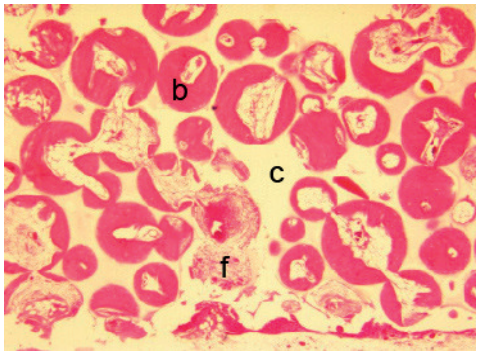
Scanning electron microscopy image of a pore of the generated construct following in vitro culture. The ceramic pore is filled with a stromal-like tissue, consisting of a 3D-network of heterogeneously shaped cells and extracellular matrix.

TISSUE ENGINEERING

Bone tissue engineering based on three-dimensional (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. 2003], nucleated cells freshly isolated from human bone marrow aspirates can be seeded onto 8 mm x 4 mm disks made of 100% hydroxyapatite (Finceramica Faenza, Italy). 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. 2005].

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. 2005]. 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, pores inter-connectivity, mechanical properties, material composition and micro architecture) and the cell-scaffold interactions during the in vitro 3D-culture.



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

Three dimensional inner architecture of porous ceramic scaffolds and attached autologous bone marrow stromal cells (BMSC) are visualized by 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 next step of this work is to analyse the visualization of the scaffold in order to find average pore size and interconnectivity, by using advanced computer vision tools like component labeling.

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