Functional Bone Tissue Engineering based on dynamic 3D-cultivation

Stefanie Röker1, Kirstin Suck1, Solvig Diederichs1, Stefanie Böhm1, Anja Peterbauer2, Birgit Weyand3, Thomas Scheper1, Martijn van Griensven2, Cornelia Kasper1

1Institut für Technische Chemie, Leibniz Universität Hannover, Callinstr. 3, D-30167 Hannover
2Ludwig Boltzmann Institut für experimentelle und klinische Traumatologie, Donaueschingenstr 13, A-1200 Wien
3Medizinische Hochschule Hannover, Plastische, Hand- und Wiederherstellungschirurgie, Carl-Neuberg-Straße 1, D-30625 Hannover

Introduction
The principle of Tissue Engineering is to build artificial replacements by means of vital components. In order to achieve a functional graft, applicable cells were seeded on three-dimensional scaffolds and expanded in vitro. The common used cell culture techniques generate cell layers, but it is not possible to create a three-dimensional, functional multilayer cell structure on the surface of a cell culture dish. Therefore, three dimensional scaffolds are necessary, which provide a specific environment and architecture for the formation of the tissue. In this study, we cultivated osteoblastic precursor cells, mesenchymal stem cells and primary osteoblasts on a zirconium dioxide ceramic with regard to their osteogenetic differentiation under static and dynamic conditions. The cell cultivation under dynamic conditions was performed in a complete platform for 3D cell cultivation consisting of a rotating bed system Z®RP in a GMP compatible breeder and a control unit allowing a GMP conform documentation and evaluation.

Material

The Z®RP system used in this study is a rotating bed reactor with a perfusion mode. The rotating bed is activated by a magnetic drive without any contact to the scaffolds. The scaffolds were moved through the cell culture medium and the overlay atmosphere alternately. The reactor is placed into a breeder, which provides a sterile and tempered atmosphere. Sponceram® is a macroporous zirconium dioxide based ceramic which is additionally microporous.

Osteoblastic precursor cells

MC3T3-E1 cells were seeded onto 8 Sponceram® discs. Four discs were cultivated in one Z®RP system for 20 days. The other four were cultivated in a second system which was changed to osteogenic BMP-2 containing medium on day 11. Glucose consumption was monitored during the cultivation. Extracellular matrix calcification (von Kossa/Alizarin red) and cellular morphology (SEM) were analysed after the cultivation. Static cultivation was used as reference:

Mesenchymal stem cells

Human mesenchymal stem cells derived from fat tissue were seeded onto Sponceram® and Sponceram®/HA (hydroxyapatite coated) discs and were cultivated in the rotating bed system Z®RP for 47 days using osteogenic differentiation medium (containing dexamethasone, β-glycerolphosphate, ascorbic acid). The continuously increasing consumption of glucose and production of lactate show good cell proliferation. Von Kossa (A) and Alizarin red staining (B) show calcification on the Sponceram matrices. Potentially the intensity of both stainings is higher on uncoated Sponceram. The SEM pictures (Sponceram®/HA above, Sponceram® beneath) show tissue like structures on both ceramic materials.

Primary osteoblasts

Human primary osteoblasts were seeded onto Sponceram® and hydroxypatite (HA) coated Sponceram® which were cultivated in the Z®RP system for 26 days. The cultivation was proceed using osteogenic differentiation medium (containing dexamethasone, β-glycerolphosphate, ascorbic acid). Glucose consumption was monitored during the cultivation. Extracellular matrix calcification (von Kossa/Alizarin red) and cellular morphology (SEM) were analysed after the cultivation. A,B: Sponceram® C,D: Sponceram®/HA.

Conclusions
In summary, with dynamic cultivation we achieved extensive cell proliferation and differentiation of osteoblastic precursor cells, mesenchymal stem cells and primary osteoblasts along the bone lineage. Tissue like structures with fibrous (collagen) and globular (mineral) extracellular matrix components stained positive for calcium, the main bone characteristic. The results were confirmed by PCR analyses which revealed bone specific markers (data not shown).

Acknowledgement
BMP-2 was kindly donated by Professor Sebald, Würzburg, Germany. Sponceram® discs were provided by Zellwerk, Oberkrämer, Germany. The human mesenchymal stem cells were contributed by Red Cross Blood Transfusion Service of Upper Austria, Vienna.