Institut für Technische Chemie
der Universität Hannover

Partner in der Forschung

Institut für Technische Chemie, Callinstr. 3, 30167 Hannover

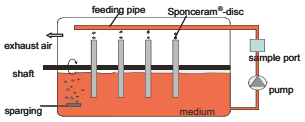
Application of a new rotating bed reactor system for cultivating 3D bone constructs

Kirstin Suck[1], Larissa Behr[1], Martijn van Griensven[2], Hans Hoffmeister[3], Thomas Scheper[1], Cornelia Kasper[1]

[1]*Institut für Technische Chemie der Universität Hannover, Callinstr. 3, 30167 Hannover, Germany*
[2]*Ludwig Boltzmann Institut für experimentelle und klinische Traumatologie, Donaueschingenstr. 13, 1200 Wien, Austria*
[3]*Zellwerk GmbH, Ziegeleistr. 7, 16727 Eichstädt, Germany*

Abstract

Aim of the project is the development and testing of a rotating-bed bioreactor for the generation of bone tissue. Large bone defects caused by tumors, infectious diseases or trauma result in a medical need for bone regeneration. The principle of bone tissue engineering is to seed osteoblasts, precursor or stem cells onto an appropriate 3D matrix and to cultivate the cell seeded scaffold in vitro in a suitabel biorector system. The generated tissue can be implantated into the defect of the patient.
During the differentiation of bone tissue different osteoblastic markers (e.g alkaline phosphatase AP) are expressed. Finally, the cells are embedded in the extracellular matrix and begin to mineralise by depositing mineral along and within collagen fibrils. The suitability of two different 3D macroporous scaffolds (Sponceram® and Sponceram®/HA) for the generation of bone tissue was investigated under static and dynamic conditions.

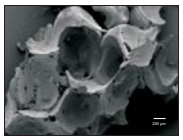


Sponceram® scaffolds

Sponceram® disc for bioreactor cultivations

Technical data of 3 different types of Sponceram®

	Sponceram® 20 - 90	Sponceram® 30 - 90	HA 90 - 90
Porosity	coarse	fine	fine
Pore size [µm]	900	600	600
Surface area (BET) [m^2/g]	1.4	2	2
Surface area total [m^2/disc]	14	14	14
Thickness [mm]	3	3	3
Diameter [mm]	65	65	65
Density [g/ml]	0.9	0.7	0.7



SEM of Sponceram® scaffold

Material and Methods

Scaffolds: Macroporous ceramic Sponceram®
Cell seeding: Scaffolds were incubated for 24 h in medium at 37°C, 5 % Co_2. Either primary osteoblasts or MC3T3-E1 cells were seeded on each scaffold in 96-well plates for 30 min at gentle stirring.
Three different media were used:
Standard medium: DMEM, 10 % FCS, antibiotics
Differentiation medium: Standard medium + 1 µM dexamethasone, 10 mM b-glycerol-phosphate, 50 µg/ml ascorbic acid
Differentiation medium + BMP-2 (10 ng/ml)
Cell viability was assayed using **MTT-test**.
Alkaline phosphatase (AP) was determined by an assay based on the hydrolysis of p-nitrophenyl phosphate to p-nitrophenol.

Pre-screening (static condition)

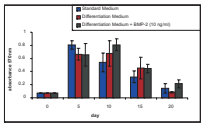
Samples of Sponceram® of approximately 3 mm x 3 mm x 4 mm were preconditioned for 24 h in cell culture medium at 37°C, 5 % CO_2. Subsequently, 1.5 x 10^4 MC3T3-E1 cells in 80 µl medium were seeded on each scaffold in 96-well dishes for 30 min at gentle stirring at 37°C, 5 % CO_2. Non attached cells were removed and the wells were filled up with 200 µl medium. The cultivations were performed for 20 days.
The cells were cultivated in three different media: standard medium, differentiation medium and differentiation medium containing BMP-2. The effect of the media composition on cell viability and the differentiation process was investigated and analysed.

BIOSTAT® B plus RBS 500

Bioreactor cultivation (dynamic conditions)

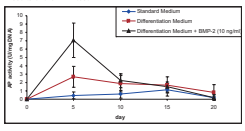
The BIOSTAT® B plus RBS 500 was designed as a multi-purpose high density cell culturing device for anchorage-dependent cell lines and primary cells.
In this study the bioreactor was equipped with 4 Sponceram® carrier discs for each cultivation. Cell inoculation was carried out with a total volume of 2 ml cell suspension/disc with a cell density of $1 \cdot 10^7$ cells per disc. To allow adhesion onto the Sponceram® surface the reactor was filled with 300 ml of differentiation medium 30 min after cell inoculation. The bioreactor features a unique technology for improved oxygen and nutrient supply through the alternating exposure to medium and gas phase. The cultivation was performed at 37°C, 2 rpm and a pH of 7.3 for 25 days.

MC3T3-E1



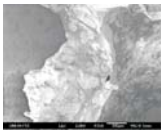
Results of the MTT-test

Results for the cell viability (MTT-test) of MC3T3-E1 cells cultured on Sponceram® in standard medium, differentiation medium and BMP-2 medium show a high cell viability during the first ten days. The cell viabilty attained a plateau phase followed by a decrease after 10 days due to high confluence on the scaffold.



Results of the AP-activity

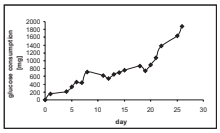
The differentiation process of the preosteoblastic MC3T3-E1 on Sponceram® was analysed by the determination of the early osteogenic marker alkaline phosphatase (AP). The highest enzyme activity was achieved at day 5 in BMP-2 medium due to the differentiation induction of the growth factor.



SEM of MC3T3-E1 on Sponceram®

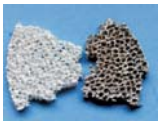
Scanning electron micrographs (SEM) show the cell morphology of MC3T3-E1 cells cultured on Sponceram®. First experiments under static conditions in 96-well dishes display that the cells grew well inside the macroporous structure of Sponceram® showing a cuboid morphology of osteoblasts-like cells. Cells were cultured for 10 days. They grew as a network having intercellular contacts to the surrounding cells.

Primary Osteoblasts



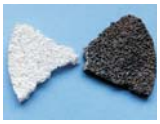
Glucose consumption during the bioreactor cultivation

During the bioreactor cultivation the cell growth of the osteobalsts was monitored by the determination of glucose consumption using the YSI 2700 (Yellow Springs Instruments, USA). Cells were cultivated on Sponceram® and Sponceram®/HA using differentiation medium. Total glucose consumption after 25 days: 14.05 g.

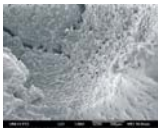


Von Kossa staining on Sponceram

The mineralisation of the extracellular matrix (ECM) was qualitatively determined by histochemical staining according to von Kossa. In each picture scaffolds are shown after bioreactor cultivation for 25 days (right) in comparison to the control matrix (left). The dark staining indicates the presence of mineralised ECM.



Von Kossa staining on Sponceram/HA



SEM of Sponceram®

After bioreactor cultivation of the primary osteoblasts a dense layer of cells and parallel structures of ECM fibrils can be observed in the SEM pictures. The ceramic material is no longer visible. Moreover, the formation of mineral nodules on the surface can be identified.

SEM of Sponceram®/HA

Conclusion

In summary, this study demonstrated that the newly developed ceramic material Sponceram® is an appropiate scaffold for the cultivation of MC3T3-E1 cells. The macroporous structure of the scaffold contributed to a fast cell attachment and proliferation. The ultimate shape of the 3D structure supports the differentiation process of preosteoblastic cells even in the absence of BMP-2. The rotating-bed bioreactor BIOSTAT® Bplus RBS features a unique technology for improved oxygen and nutrient supply for the cells through the alternating exposure to medium and gas phase. Therefore, the BIOSTAT® Bplus RBS equipped with Sponceram® discs provides an optimal environment for bone tissue generation. Primary osteoblasts were successfully cultivated on Sponceram® and Sponceram®/HA for 25 days in the bioractor system. The analyses revealed a bone tissue-like generation of a fibril structured mineralised ECM.

Acknowledgement
The authors like to thank Dr. A. Feldhoff and F. Steinbach (University of Hannover) for the SEM pictures und Prof. W. Sebald for kindly donating BMP-2.