Institut für Technische Chemie
der Universität Hannover
Institut für Technische Chemie, Callinstr. 3, 30167 Hannover

Partner in der Forschung
Universität Hannover

Development of Bone Constructs Taking into Account Physiological Aspects

Kirstin Suck[1], Cornelia Hildebrandt[1], Cornelia Kasper[1], Solvig Diederichs[1], Martijn van Griensven[2]

[1]*Institut für Technische Chemie der Universität Hannover, Callinstr. 3, 30167 Hannover*
[2]*Ludwig Boltzmann Institut für experimentelle und klinische Traumatologie, Donaueschingenstr. 13, 1200 Wien, Österreich*

Introduction

One main objective in bone tissue engineering is the construction of precise scaffolds in order to support and guide cell growth and differentiation. These scaffolds should substitute the extracellular matrix (ECM) characteristics and should form the desired structure with similar physical properties to bone tissue. Moreover, to ensure proper function of the tissue mechanical loading has to be applied. Mechanical strain mimics physiological environment and thus supports the differentiation process.

In this work, osteoblast like SAOS-2 cells and bone marrow stromal cells (BMSCs) were cultured onto ceramic scaffolds. The viability on the scaffolds was investigated using MTT-Test and the differentiation was analyzed by alkaline phosphatase assay. Additionally, a cyclic mechanical stress was applied to maintain and enhance the differentiation and physiological properties of the cells. Extracellular matrix protein levels were detected and semi-quantified using RT-PCR. Activation of signal transduction proteins was measured using the western blot technique.

Mechanical strain experiments

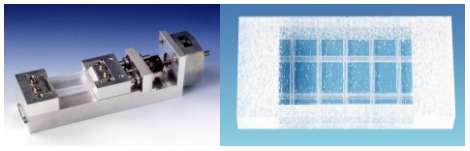
Human bone marrow aspirates were obtained during routine orthopaedic surgical procedures involving exposure of the iliac crest. BMSCs were isolated using a density gradient. Predifferentiated second passage BMSCs and SAOS-2 cells (medium: DMEM 10% FCS, antibiotics, 1 µM dexamethasone, 10 mM -glycerolphosphate, 50 µg/ml ascorbic acid, 37°C, 5% Co_2) respectively were seeded on silicone dishes prepared of a two component silicone resin. Serum concentration was reduced to 1 % for 24 h to align the cells into the G_0 cell cycle phase. The cells on the silicone dishes were strained longitudinally with a frequency of **1 Hz; amplitude of 5 % for 3× 8 h**. For analysis, cells were scratched off the silicone dishes. Extracellular matrix protein levels (fibronectin, collagen I + III) were detected and semi-quantified using **RT-PCR**. Activation of signal transduction proteins (p38, ERK, JNK) was measured using the **western blot** technique. Control cells were seeded onto siliconedishes but not mechanically strained.

Experiments with Sponceram®

Scaffolds: macroporous ceramic Sponceram®

Cell seeding: scaffolds were incubated for 24 h in DMEM 10% FCS, antibiotics, +/- differentiating conditions (1 µM dexamethasone, 10 mM -glycerolphosphate, 50 µg/ml ascorbic acid) at 37°C, 5% CO_2. An excess of osteoblast-like SAOS-2 cells or BMSCs was seeded on each scaffold in 96-well plates for 30 min at gentle stirring. Before each of the following tests the scaffolds were placed into a new 96-well plate. Since it was not possible to estimate the attached number of cells on the scaffolds, the first measurements were performed directly after cell seeding. Cell metabolism was assayed using **MTT-test**. Total protein content was measured using Micro **BCA** assay. **Alkaline phosphatase (AP)** was determined by an assay based on the hydrolysis of p-nitro-phenyl phosphate to p-nitrophenol.

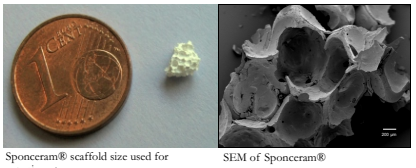
Strain chamber



Cell staining system | Silicone dish

The cell stretching system consists of rectangular, elastic silicone dishes in which the whole dish, not only the cell culture surface was deformable.

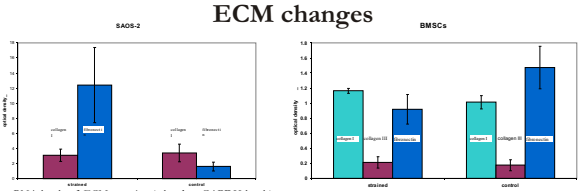
Sponceram® scaffolds



Sponceram® scaffold size used for experiments | SEM of Sponceram®

Sponceram® scaffolds consist of a macroporous structure.

ECM changes



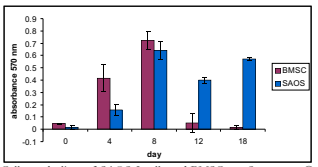
mRNA levels of ECM proteins (related to GAPDH levels) in SAOS-2 cells measured by RT-PCR. Values represent the mean of 5 samples, cultured under described conditions.

Fibronectin level in SAOS-2 cells decrease vigorously by mechanical strain while the collagen I level remains unaffected. Scar tissue specific collagen III was not detectable proving no harm for the strained cells.

mRNA levels of ECM proteins (related to GAPDH levels) in BMSCs measured by RT-PCR. Values represent the mean of 5 samples, cultered under described conditions.

Bone markers collagen I and fibronectin show small reaction to long term mechanical strain: the collagen I levels tends to decrease, the fibronectin level tends to increase. Collagen III concentration is low and remains unaffected by mechanical strain proving no harm for the cell.

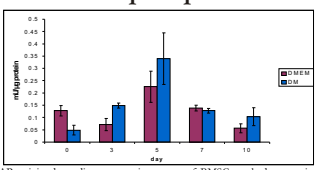
Cell metabolism



Cell metabolism of SAOS-2 cells and BMSCs on Sponceram® scaffolds over a time period of 18 days. Values represent the mean of 5 samples of cultured scaffolds +/- SD.

Cell metabolism assay shows a fast cell growth of both cell types during the first 8 days. Due to high confluence on the scaffolds, the BMSCs viability decrease rapidly after 10 days. In comparison, the viability of SAOS-2 cells remains more stable.

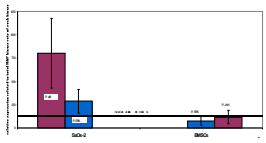
Alkaline phosphatase



AP activity depending on protein content of BMSCs on hydroxyapatite coated Sponceram® over a time period of 15 days. Values represent the mean of 5 samples of cultured scaffolds +/- SD.

BMSCs cultivated on hydroxyapatite coated Sponceram® show an increase in alkaline hosphatase activity with a maximum after 5 days in DMEM and differentiation medium. As expected the AP activity under differentiation conditions is higher.

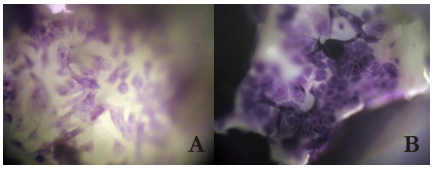
Signal transduction



Levels of activated MAP kinases related each to the respective total kinase amount and -actin. Values represent the mean of 5 samples, cultered under described conditions

Mechanical strain increased p38 activation in SAOS-2 cells 8fold and ERK activation 3fold, while ERK and JNK activation in BMSCs slightly decreased. JNK activation in SAOS-2 cells and p38 activation in BMSCs could not be detected.

Microscopy



Toluidin-Blue staining of SAOS-2 (A) cells and BMSCs (B) cultured on Sponceram® after 10 days.

Conclusion

The results obtained in this work showed that Sponceram is an appropiate scaffold for bone tissue engineering. Moreover, the application of mechanical strain accelerates the differentiation of BMSCs. For further experiments a combination of the used scaffolds and mechanical strain is planned to induce specific osteogenic differentiation.

Acknowledgement

SAOS-2 cells were provided by Cell Culture Systems and Services , Hamburg (Germany). Sponceram was purchased from Zellwerk, Oberkrämer (Germany).