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Adipose Tissue Derived Stem Cells for Bone Tissue Engineering

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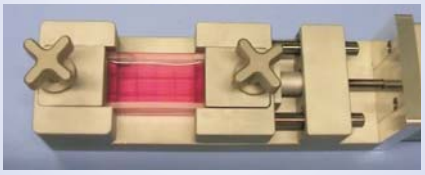
Introduction: Mesenchymal stem cells (MSC) are the focus of various different projects in the area of stem cell research, tissue engineering and regenerative medicine.MSCs can be obtained from multiple tissues including bone marrow, peripheral blood, and adipose tissue. Currently, bone marrow is the main source for MSCs, but bone marrow procurement procedures may be painful, frequently requiring general or spinal anesthesia and may yield low numbers of MSCs upon processing. Therefore, adipose tissue seems to be a beneficial alternative, since it is easy to obtain and it is capable of yielding cell numbers substantial enough to obviate extensive expansion in culture. However, the presence of MSCs in adipose tissue has been discovered only a few years ago, and adMSCs are therefore not yet characterised sufficiently. In this research project, adMSCs are analysed for their applicability in bone tissue engineering. For bone tissue engineering various scaffold materials have been described. A three dimensional, interconnected porous structure is currently supposed to be most suitable for bone regeneration. The most frequently used materials for bone tissue engineering are calciumphosphates and hydroxyapatites. Recently, mechanical stimulation has entered the field of tissue engineering as a tool for promoting the development of a number of tissue types including bone *in vitro*. Mechanical strain has been shown to promote osteogenesis of bone marrow derived MSCs *in vitro*. Additionally, mechanical strain is supposed to accustom cells to their future mechanically active environment.

Materials and methods

Cell culture: Mouse preosteoblast cells MC3T3-E1 and human adipose tissue derived mesenchymal stem cells (adMSCs) were cultured in DMEM + 10% FCS and antibiotics. Osteogenic differentiation was performed with standard medium supplemented with 10 nM dexamethasone, 0.3 mM ascorbic acid and 10 mM beta-glycerolphosphate.

Mechanical strain experiments: Cells were grown on a flexible silicone dish. The concentration of calf serum was reduced to 1% for 24 hours in order to align the majority of cells into the G_0 phase of the cell cycle. Afterwards, the cells in the silicone dishes were exposed to a cyclic longitudinal strain at a frequency of 1 Hz with 5% strain amplitude.
Control cells were cultivated on silicone dishes without any mechanical stimulation.

Left: Flexible silicone dish with culture area of 2.3 x 5 cm.
Right: Silicone dish with Matriderm® bottom.

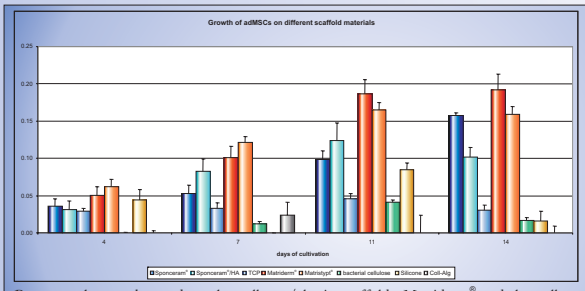


Mechanical stimulation device to strain a silicone dish with adherent growing cells.

Scaffold materials:
Sponceram®: consists of doped ceramic material (ZrO_2) developed by Zellwerk GmbH (patent pending)
Sponceram®/HA: hydroxyapatite coated Sponceram®
TCP: tricalcium phosphate
Matriderm®: consists of collagen I / elastin developed by Dr. Suwelack Skin&Health Care AG
Matristypt®: consists of collagen I, developed by Dr. Suwelack Skin&Health Care AG
Coll/Alg: consists of collagen I / alginate developed by Dr. Suwelack Skin&Health Care AG
Silicone: addition-curing two-component silicone rubber

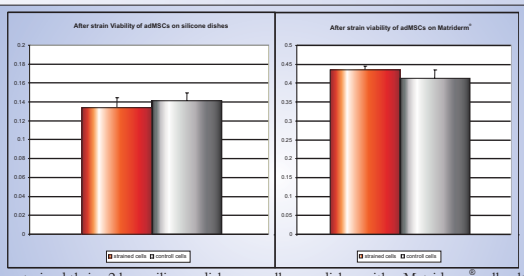
Cell metabolism: After strain cell viability was monitored via MTT-assay.

Osteogenic markers were semi-quantified with RT-PCR.

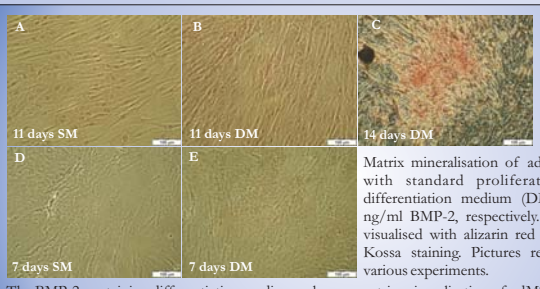


AdMSCs were cultured on different scaffold materials. Cell viability was monitored for 2 weeks with the MTT-assay.Values are given as mean of 5 samples +/- SEM.

Best growth was observed on the collagen/elastin-scaffold Matriderm® and the collagen scaffold Matristypt®. Growth on Sponceram® and hydroxyapatite coated Sponceram® as well as silicone was still good while on TCP, bacterial cellulose and collagen alginate only poor cell proliferation was observed.

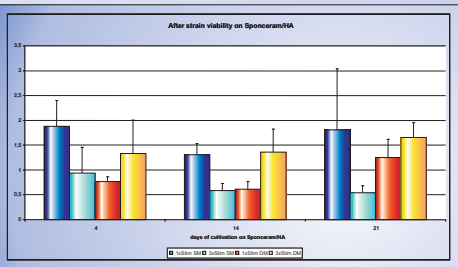


AdMSCs were strained thrice 2 h on silicone dishes as well as on dishes with a Matriderm® cell culture surface. Cell viability was observed with the MTT-assay. Values are given as mean of 5 samples +/- SEM.
Cell viability was not affected by mechanical strain, neither on the silicone dish nor on the collagen/elastin surface of Matriderm®.



Matrix mineralisation of adMSCs. Cells were cultured with standard proliferation medium (SM) and differentiation medium (DM) supplemented with 20 ng/ml BMP-2, respectively. Matrix mineralisation was visualised with alizarin red staining (A-C) or the Von Kossa staining. Pictures represent typical results of various experiments.

The BMP-2 containing differentiation medium enhances matrix mineralisation of adMSCs as has been shown both with the alizarin red and the Von Kossa staining. After 14 days of cultivation even so called mineralisation nodes were detected (C).



Values are given as mean of 3 samples +/- SEM and in comparison to the control cell viability.

Cells were strained once 2 h and thrice 2 h, respectively and subsequently cultivated on Sponceram®/HA for up to 4 weeks. Repeated strain can have different effects than singular strain. But effects are dependant on the cultivation medium.

Conclusions and Outlook

Mesenchymal stem cells derived from fat tissue are a promising cell source for bone tissue engineering. They show good growth on the most important scaffold materials hydroxyapatite and type I collagen. Furthermore, it has been shown that mechanical strain, which is known to be of major importance for tissue engineering applications, does not have any detrimental effects on adMSCs. More importantly, mechanical strain can influence cell viability and protein expression dependant on strain duration (data not shown). The first attempt to combine mechanical strain and cultivation on a three-dimensional scaffold were succsessful. It was even shown that different time schemes of mechanical strain can influence cells differently. Further experiments will include a DNA microarray comparing the osteogenic differentiation status of cells cultured in media with or without BMP-2. Moreover, the influence of mechanical strain on the expression of osteogenic markers will be investigated more deeply. The combination of mechanical strain and cultivation on scaffolds needs to be investigated further, since still little is known about their influence on cell metabolism.

EXPERTISSUES

Acknowledgment

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