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Mechanical straining of adipose tissue derived mesenchymal stem cells for application in bone tissue engineering

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Introduction: The engineering of functional bone constructs *ex vivo* is a rapidly growing branch in the field of tissue engineering. Bone constructs generally comprise cells and a scaffold providing a supportive framework for the cells to grow as well as mechanical stability. For bone tissue engineering mesenchymal stem cells (MSCs), which can be differentiated into bone cells, are an attractive cell source. The isolation of MSCs from fat tissue has been described only recently, and therefore, these cells (adMSCs) are not characterized sufficiently yet.

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.

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.

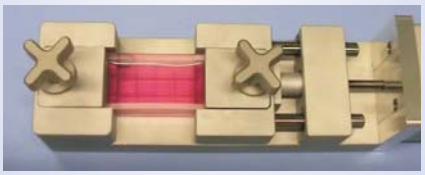
Materials and methods

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.

Scaffold material:

- *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
- *Silicone:* addition-curing two-component silicone rubber

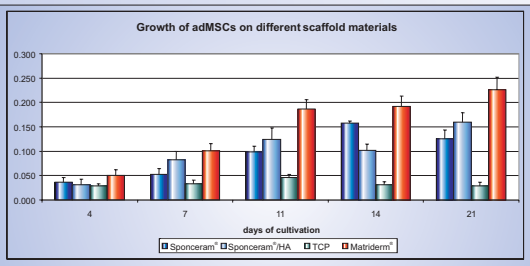
Left: Flexible silicone dish with culture area of 2.3 x 5 cm.
Right: Silicone dish with Matriderm® bottom.



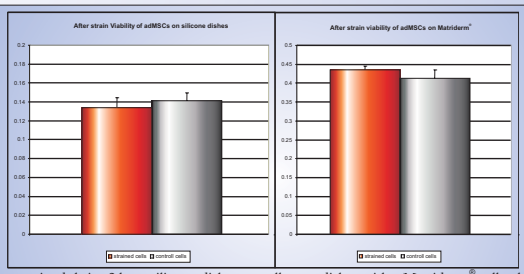
Mechanical stimulation device to strain a silicone dish with adherent growing cells.

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.

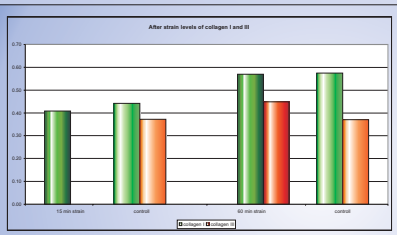
Cell metabolism: After strain cell viability was monitored via MTT-assay.

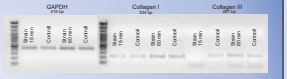


AdMSCs were cultured on different scaffold materials. Cell viability was monitored for 3 weeks with the MTT-assay. Values are given as mean of 5 samples +/- SEM.
Best growth was observed on the collagen/elastin-scaffold Matriderm®. Growth on Sponceram® and hydroxyapatite coated Sponceram® was still good while on TCP only little cell proliferation was observed.



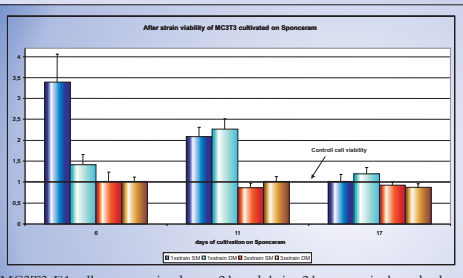
AdMSCs were strained thrice 2 h on silicone dishes as well as on dishes with a Matriderm® cell culture surface. Cell viability was observed with the MTT-assay. Values are given as mean of 5 samples +/- SEM.
Cell viability was not affected by mechanical strain, neither on the silicone dish nor on the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





For RT-PCR 5 samples of strained or controll cell RNA were pooled. Semi-quantitative values are given compared to GAPDH levels of the respective sample.

AdMSCs were strained on silicone dishes for 15 min and 60 min, respectively. Type I and type III collagen levels as well GAPDH were determined using semi-quantitative RT-PCR. Type I collagen is constitutively expressed by adMSCs and levels are not affected by the applied strain. Type III collagen a marker for scar tissue and hence, for cellular damage is strongly decreased after 15 min strain but slightly increased after 60 min strain. Mechanical strain therefore induces cell repair mechanisms in a time-dependant way.



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

MC3T3-E1 cells were strained once 2 h and thrice 2 h, respectively and subsequently cultivated on Sponceram®/HA for up to 17 days. The cell viability was initially increased by singular strain but adjusts with time to controll cell viability. On the contrary, repeated strain did not affect cell viability on the scaffold.

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. The first attempt to combine mechanical strain and cultivation on a three-dimensional scaffold with mouse preosteoblasts as model cell line were succsessful. It was even shown that different time schemes of mechanical strain can influence cells differently. 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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