Leibniz Universität Hannover

Partner in der Forschung

Institut für Technische Chemie, Callinstr. 3, 30167 Hannover

Mechanical Stimulation of Adipose Tissue Derived Mesenchymal Stem Cells for Bone Tissue Engineering

Solvig Diederichs[1,2], Susanne Kall[3], Cornelia Kasper[1], Martijn van Griensven[2], Thomas Scheper[1]

[1]: Institut für Technische Chemie der Universität Hannover, Callinstr. 3, 30167 Hannover, Germany
[2]: Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Donaueschingenstraße 13, A-1200 Vienna, Austria
[3]: Klinik für Plastische, Hand- und Wiederherstellende Chirurgie, Medical School Hannover, Podbielskistraße 380, 30659 Hannover, Germany

Introduction: Mesenchymal stem cells are a widely used cell source in tissue engineering and regenerative medicine due to their ability to be expanded and differentiated easily *in vitro* as well as *in vivo*. While the presence of those MSCs in bone marrow has been known since the 1980s they were discovered in adipose tissue only in 2001. In the meantime, both similarities and differences between BMSCs and adMSCs have been described but still the cells have not been characterized sufficiently.

Mechanical stimulation has become a substantial tool in tissue engineering to accustom cells to their future physically active environment and even to stimulate differentiation of stem and progenitor cells. Therefore, adMSCs were subjected to a cyclic strain with 5 % elongation and a frequency of 1 Hz. Cell viability and osteogenic differentiation were examined in reliance of different time schemes.

Materials and Methods

Cell Culture: Adipose tissue derived mesenchymal stem cells (adMSCs) were cultured with proliferation medium NM (DMEM, 10% FCS, antibiotica) for up to three passages. Experiments were conducted with third to fourth passage adMSCs.

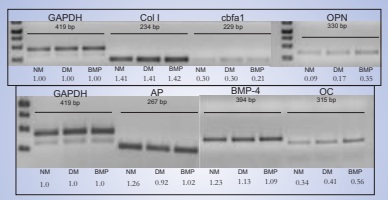
Osteogenic Differentiation: To differentiate the cells to the ostegeonic lineage, adMSCs were cultivated with differentiation medium DM (proliferation medium + 10 nM dexamethasone + 0.3 mM ascorbat + 10 mM beta-glycerolphosphate) or DM supplemented with 10 ng/ml BMP-2 for 7 days. Expression rates of several osteogenic markers were examined via RT-PCR.

Scaffold Materials: The growth of adMSCs on different scaffold materials was monitored via MTT-assay. Sponceram® is a ZrO_2 based porous ceramic, which is also available with a hydroxyapatite (HA) coating produced by Zellwerk GmbH, Eichstädt, Germany. ZrO_2 based materials have been used extensively in dental surgeries.

Beta-tricalcium phosphate (TCP) like HA is a common scaffold material in bone tissue engineering. Porous TCP granules (chronOS™) were obtained from Mathys Medical Ltd (Bettlach, Switzerland). For the MTT assay, 10.000 cells were seeded onto each scaffold and cultured in proliferation medium for up to 21 days.

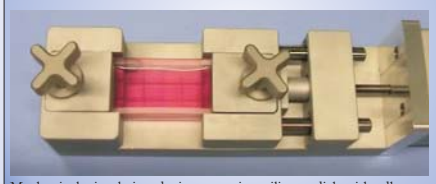
Mechanical Strain Experiments: AdMSCs were precultured with DM for 7 days before seeding onto a flexible silicone dish. The concentration of calf serum was reduced to 1% for 24 hours prior to the experiment in order to align the majority of cells into the G_0 phase of the cell cycle. Afterwards, the cells in the silicone dishes were exposed to a cyclic longitudinal strain at a frequency of 1 Hz with 5% strain amplitude.

Results

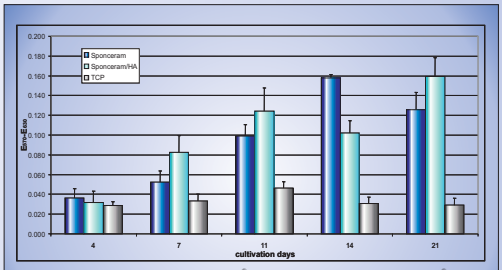


AdMSCs were cultured with NM, DM or BMP-2 containing medium. RT-PCR bands revealed osteogenic markers like collagen I, cbfa1, osteopontin, alkaline phosphatase, BMP-4 and osteocalcin. Band intensity was related to the housekeeping enzyme GAPDH. Interestingly, the cells expressed all the bone markers even without any osteogenic stimuli. Only three markers were found to be regulated depending on the medium: osteopontin and osteocalcin, as well as alkaline phosphatase.

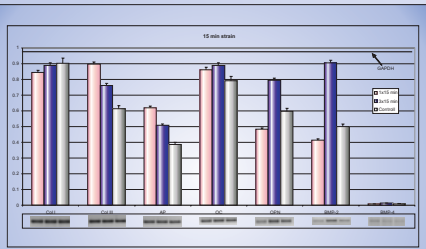
Flexible silicone dish with culture area of 2.3 x 5 cm.



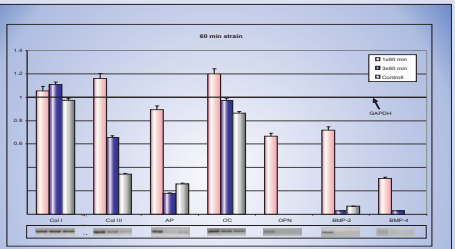
Mechanical stimulation device to strain a silicone dish with adherent growing cells.



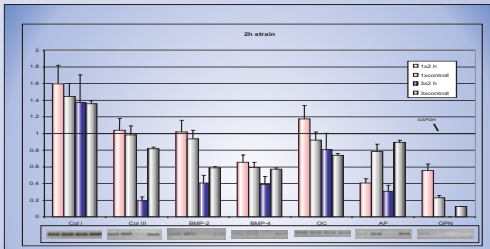
The growth of adMSCs on pure Sponceram®, on hydroxyapatite coated Sponceram® and on beta-Tricalciumphosphate TCP was monitored via MTT assay over three weeks. Best cell viability / proliferation was obtained on hydroxyapatite, only slightly lower viability on pure Sponceram®. On TCP, adMSCs showed no notable proliferation.
Values are given as mean of 5 samples +/- SEM.



AdMSCs were strained once for 15 min and thrice 15 min with 30 min intermission. Subsequently, bone marker expression was examined via RT-PCR. PCR was performed with 5 pooled samples, error bars represent the SEM of GAPDH PCRs performed with individual samples. Several bone markers were detected. Short time strain of once 15 min did not have distinct effects on osteogenic markers but enhanced collagen III expression indicating cell damage. A repitition of 15 min strain had stronger effects on osteogenic marker expression. The decline of collagen III expression suggests accustomization to the strain.



AdMSCs were strained once for 60 min and thrice 60 min with 2 h intermission. Subsequently, bone marker expression was examined via RT-PCR. Several bone markers were detected and standardized to GAPDH expression. Once 60 min strain had stronger effects on osteogenic marker expression than repeated strain of 60 min. But again, collagen III expression indicated an evolving strain tolerance.



AdMSCs were strained once for 2 h and thrice 2h with 4 h intermission. Subsequently, bone marker expression was examined via RT-PCR. Several bone markers were detected and standardized to GAPDH expression. Once 2 h strain had only little effect on osteogenic marker expression. Only osteopontin and alkaline phosphatase levels were affected. Repeated 2 h strain did not have more effects on osteogenic markers but revealed strain tolerance indicated by very low collagen III expression.

Conclusions and Outlook

Adipose tissue derived mesenchymal stem cells were shown to be excellently applicable in bone tissue engineering. They proliferate on hydroxyapatite. Their good osteogenic differentiation capacity was demonstrated on mRNA level. For a more detailed understanding of the osteogenic differentiation of these cells a DNA microarray experiment with these cells is aspired. Mechanical strain was shown to have different effects on adMSC differentiation depending on strain duration and repetition. Strongest effects were achieved with thrice repeated 15 min strain and with once 60 min strain. Strain repetition resulted in decreased collagen III level. Collagen III is typical for scar tissue, thus, indicating the cells initiating repair mechanisms. Therfore, decreasing collagen III expression can be interpretated as development of strain tolerance.
So far, only short time experiments have been performed. Longer strain experiments are planned. Their influence on the osteogenic differentiation of adMSCs is aspired to be monitored not only with RT-PCR but also with quantitative PCR and on protein level.
Nutrient supply especially on 3D scaffolds is heterogeneous in static cell culture leading to reduced growth rates or even death of the tissue on the scaffold. Thus, specifically customised bioreactor systems have been developed to control tissue growth. In this work cultivation of adMSCs in a rotating bed system bioreactor is aspired in order to enhance the growth of a biologically active and reproducible tissue.

Acknowledgments

EXPERTISSUES

This study was supported by the European project NoE EXPERTISSUES (NMP3-CT-2004-500283).

Sponceram® and hydroxyapatite coated Sponceram® were a kind gift of Prof. Hoffmeister from Zellwerk GmbH. BMP-2 was a kind gift of Prof. Sebald, Würzburg, Germany.