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Osteogenic differentiation of MG-63 cells induced by mechanical strain in a three dimensional collagen scaffold

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Introduction

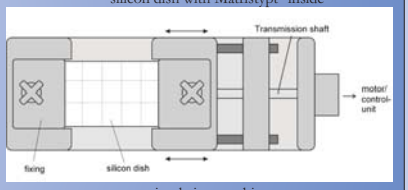
The aim of this work was to study the effect of mechanical strain on the osteogenic differentiation of MG-63 cells in a three dimensional collagen scaffold. The cells were stimulated by the application of straining programms. The stimulations were performed once and thrice. Between the stimulations a regeneration time which took twice of the stimulation time was rested. After the experiments the viability of the cells was determined by an MTT assay. Furthermore, a PCR was performed to investigate the expression of bone markers and the activity of alkaline phosphatase was examined by an AP-activity test. Moreover histological stainings were performed.

Materials and Methods

The stimulations were performed in a stimulation machine which expanded the silicon dishes with the cells inside. The experiments were performed by 1 Hz and 5 % extension amplitude. The cells were stimulated with mechanical extension but they were also set out to fluid flow shear stress due to the medium flow. The cells were stimulated for 15 minutes, 60 minutes, two hours, four hours and eight hours. The stimulations were performed once and thrice. Furthermore, a continuous stimulation was applied where the previous stimulations were performed in series. Parallel to the stimulations as a reference cells were cultivated on the collagen scaffold without stimulation.

Into the silicon dish a three dimensional collagen matrix (Matristypt®) was placed and was used as a stabilizing network for the cells. Furthermore the cells were supposed to differentiate in an environment which was similar to their natural surrounding.

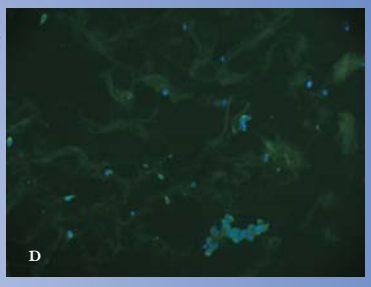
silicon dish with Matristypt® inside

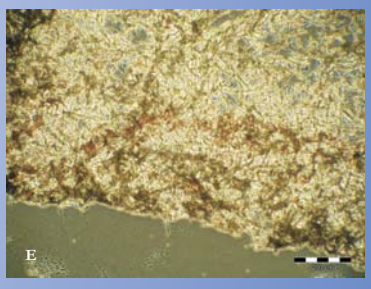


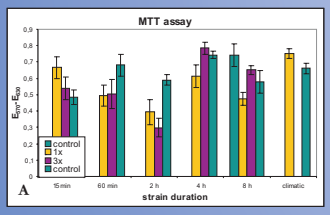
stimulation machine

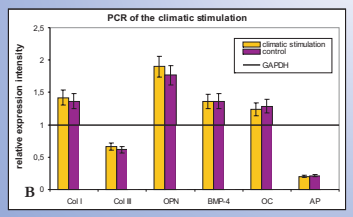
Results

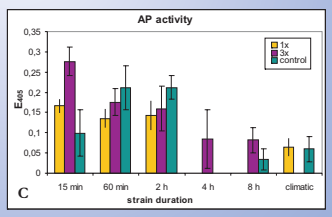
After the experiments the viability of the cells was determined by an MTT assay (A). The viability was not affected by the mechanical stimulation. The mRNA of the cells was isolated and a PCR was performed to investigate the expression of bone markers (B). The expression of typical bone markers was found. It was noticeable that each stimulation scheme had different influence on the cells. The expression of bone markers varied in the different stimulations. The activity of alkaline phosphatase was examined by an AP-activity test and was verified for almost all stimulations (C). There was no difference between the AP activities of the stimulated und unstrained cells. The synthesis of alkaline phosphatase and RUNX2 were tested with immunological staining. RUNX2 was not observed in any experiment. Alkaline phosphatase was substantiated in the most experiments (D). The mineralisation of the extracellular matrix was studied by an alizarinred/calcein-double staining (E). Especially by the threefold stimulation and the unstrained cells an explicit mineralization was recognized.











Conclusion and Outlook

The mechanical stimulations evoked osteogenic differentiation. An expression of bone markers was noticed. The synthesis of alkaline phosphatase was proved by an immunological staining and an activity test. Furthermore the mineralization of the bone matrix was proved. It was noticeable that a short time of stimulation, for example one time 15 minutes, sufficed to induce osteogenic differentiation. A long time of stimulation evoked an enhancement of the extracellular matrix. The climatic stimulation induced an adaption of the cells to the mechanical stimulation. While a stimulation with long time periods like the three time eight hour stimulation induced a high expression of Collagen III. Collagen III occurrences in scar tissue and an increased expression could be a result of a cell damage induced by mechanical stimulation.

Acknowledgement

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