



Effects of mechanical stimulation concerning the osteoblastic differentiation

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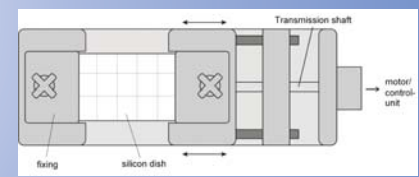
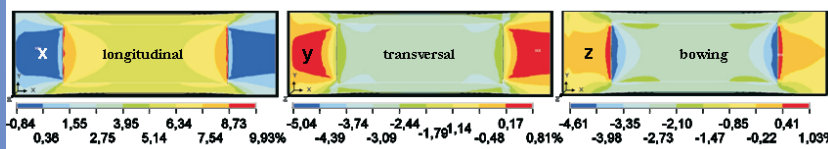
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Introduction

The aim of this work was to study the effect of a three dimensional collagen scaffold and mechanical strain on the osteoblastic differentiation of MG-63 cells. Therefore six different stimulations were performed: 15 Minutes stimulation, 60 Minutes, two hours, four hours and eight hours. Each experiment was performed once and thrice. Between the stimulations a regeneration time which took twice of the stimulation time was rested. Furthermore, a continuous stimulation was applied where the previous stimulations were performed in series. Parallel to these experiments cells were cultivated on the collagen scaffold without mechanical stimulation. After finishing the experiments the viability of the cells was checked by an MTT assay. A PCR was performed to determine the expression of bone markers and the activity of alkaline phosphatase was tested by an AP-activity test. Furthermore histological stainings were performed.

Materials and Methods

The stimulations were performed in a stimulation machine which expanded the silicon dishes with the cells on the collagen scaffold, called Matristypt[®], inside. The experiments were performed by 1 Hz and 5 % extension amplitude. In addition to the mechanical extension the cells were stimulated by fluid flow excited by the medium flow. The stimulation machines were activated with a step motor. The motors were cooled with water so that long stimulation times could be performed without overheating of the motors. With special software each stimulation machine could be actuated individually.

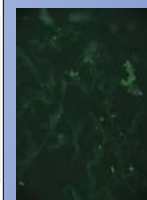
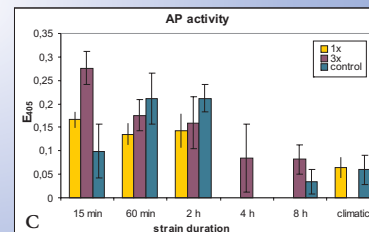
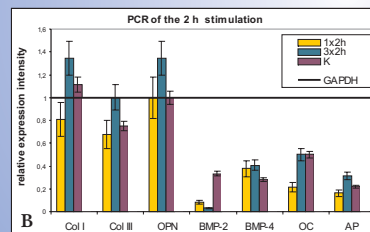
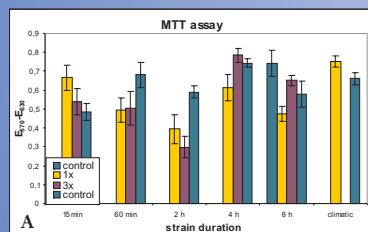


silicon dish with Matristypt[®]

stimulation machine

Results

In most instances the mechanical stimulations had no impact on the the viability of the cells (A). The expression of bone markers were determined by a PCR (B). Each stimulation scheme had different influence on the cells and 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). No difference between the AP activities of the stimulated und unstrained cells was found. The synthesis of alkaline phosphatase and RUNX2 were detected with immunological staining. Thereby RUNX2 could not verified in any case but alkaline phosphatase was substantiated in almost all experiments (D). The mineralisation of the extracellular matrix was proved with an alizarinred staining (E). An explicit mineralisation was recognized by the cells which were stimulated for three times and the unstrained cells.



D

E

Discussion and Outlook

The mechanical stimulations had no influence on the viability of the cells and evoked osteogenic differentiation. An expression of bone markers was substantiated. The synthesis of alkaline phosphatase was proved by an immunological staining and an activity test. Furthermore the mineralization of the bone matrix was verified. It was recognized that a “short time” stimulation sufficed to induce osteogenic differentiation. A “long time” stimulation evoked an enhancement of the extracellular matrix. While the climatic stimulation induced an adaption of the cells to the mechanical extension. Long periods of stimulation like the three time eight hour stimulation caused a high expression of Collagen III. This could be a hint for cell damage because Collagen III occurrences in scar tissue.

Acknowledgement

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