Co-culture of osteoblast and osteoclast like cells is influenced by mechanical stimulation

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Introduction
Mechanical straining affects bone modeling and remodeling conducted by osteoblasts and osteoclasts [Frost 1992; Eichhoff 1993; Rubin and McLeod 1994]. Osteoclast differentiation and activation are mediated by receptor activator of nuclear factor-kB ligand (RANKL), Osteoprotegerin (OPG) and macrophage stimulating factor (MCSF). All three cytokines are synthesized by osteoblasts, indicating the tight interaction of both cell types. Aim of the study was to analyze the interaction and the behavior of both cell types in a co-culture system under the influence of different mechanical stimulation durations and frequencies. We hypothesise that the biological interaction of both cell types influences the reaction of the cells to mechanical stimulation.

Material and Methods
Primary human osteoblasts (POB) were isolated from human trabecular bone from three different donors according to established protocols. The cells were cultured and expanded under standard conditions (MEM-E/Ham’s F-12 +10 % heat inactivated FCS, Glycerol phosphate, L-Ascorbic acid, Penicillin/Streptomycin). Osteoclast like cells (OCL) were isolated from peripheral blood by density gradient centrifugation and CD-14+ magnetic activated cell sorting.

Fusion assay: POB were seeded in a concentration of 3x10^5 cells per well in 24-well plates on dentine chips and cultured in MEME/HAM’s F-12 for 3 to 4 days. 5x10^5 monocytes were then added per well. Both cell types were incubated together using MEME/HAM’S F-12 for further 4 days followed by 5 days mechanical stimulation.

Resorption assay: Monocytes were cultured (3 x10^6) in 24-well plates on dentine chips for 14 days with ALPHA-MEM plus Nuclear factor-kB ligand (RANKL) (20 ng/ml) and macrophage colony stimulating factor (MCSF) (5 ng/ml). After 14 days, 3x10^4 POB were added. Further cell cultivation of both cell types in MEME/HAM’S F-12 was performed in absence of RANKL and MCSF for 3 days followed by mechanical stimulation for 5 days.

For mechanical stimulation three point bending was applied to the dentine chips as described previously [Kadow-Romacker CTO 2008]. Using a minimum of 1100 µstrain bending magnitude of the dentine chip following stimulations were performed:
1 minute with 0.1 Hz; 1 minute with 0.3 Hz; 3 minutes with 0.1 Hz; 3 minutes with 0.3 Hz; 5 minutes with 0.1 Hz.

Cell Count: Cell activity assay (AlamarBlueTM, Biozol, Germany). Tartrate-resistant acid phosphates (TRAP): positive and polynucleated (more than 3 nuclei) cells were considered as osteoclast like cells and counted (magnification 10x).

Pit resorption Assay: resorption lacunae on the dentine were stained with toluidin blue and counted (magnification 10x).

Total Protein: Coomasie (Pierce, Germany)
Cross Laps CTX: to measure collagen resorption (Nordic Bioscience Diagnostics, Denmark).
CICP: to measure collagen formation (Metra, Germany)
OPG and RANKL ELISA: (Immun Diagnostik, Germany)
The ELISA data were correlated to total protein

Statistics: ANOVA, Dunnett

Results
Neither in the fusion nor in the resorption assay were differences in the cell vitality and total protein content after mechanical stimulation measurable. In the fusion assay, however, the mechanical stimulation resulted in a significant decrease of the collagen type 1 and osteocalcin amount measurable in the supernatant (Fig. 1). The amount of RANKL and OPG was not different between the groups. No effect on the fusion behaviour of monocytes to osteoclast like cells was detectable.

In the resorption assay the stimulation for 3 minutes with 0.3Hz significantly increased the activity of osteoclast like cells as measured by the pit formation and the collagen resorption (CTX). The same stimulation resulted in an increased collagen type 1 production by osteoblast like cells (Fig. 2).

Discussion
The mechanical stimulation of the co-culture of osteblast and osteoclast like cells revealed differences in the resorption activity of fused osteoclast like cells and collagen production of osteoblasts. The decrease in collagen-1 production by osteoblast like cells was also detectable in a previous study investigating the pure osteoblast culture [Kadow-Romacker CTO 2008]. The effect seen in the co-culture resorption assay (3 min with 0.3 Hz: increased resorption and collagen 1 synthesis) was not detectable in the pure osteoclast or pure osteoblast culture [Kadow-Romacker CTO 2008]. A shorter stimulation and with less frequency, however, resulted in a decreased osteoclastic activity.

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