BMP-2, IGF-I and TGF-β1 reveal different effects on the co-culture of osteoblast- and osteoclast-like cells
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Introduction
Several studies have demonstrated the effect of growth factors on osteoblasts and osteoclasts in vitro. Due to the fact that these cell types interact and the effect of the growth factors might therefore be influenced by this interaction, aim of this study was to investigate the influence of growth factors (IGF-I, TGF-β1, BMP-2) on co-cultured osteoblast- and osteoclast-like cells.

Material & Method
Human primary osteoblast-like cells or peripheral blood mononuclear cells (PBMC) were used and cultured in two different set-ups.
1. Fusion assay: Determination of PBMC differentiation (fusion) to osteoclast-like cells in the presence of osteoblast-like cells.
2. Resorption assay: Determination of bone resorption activity of osteoclast-like cells in the presence of osteoblast-like cells. PBMC were pre-cultured for 14 days with MCSF (5 ng/ml) and RANKL (20 ng/ml) on dentine disc to stimulate osteoclastogenesis. Than osteoblast-like cells were added and co-cultured.
Both set-ups were cultured for 15 days with the different growth factors.
Following groups were investigated:
1. PDLLA (Control), 2. BMP-2 (25 µg), 3. IGF-I (25 µg), 4. TGF-β1 (5 µg), 5. IGF-I/TGF-β1 (25 + 5 µg).
PDLLA: poly(D,L-lactide) implant coating serves as a carrier for the growth factor as described previously [1].
Cell vitality: Alamar blue (Biozol, Germany)
TRAP: Fast Red Violet LB Salt (Sigma, Germany), Naphtol-AS-MX-Phosphat (Sigma, Germany) in TRAP-staining buffer (40 mM sodium acetate and 10 mM sodium tartrate dibasic dihydrate)
Resorption pits: dentin slices were stained with toluidine blue and pits were counted.
ELISA: Collagen-Type 1 (COL-1) & Osteoprotegerin (OPG), RANKL, TRAPiso-5b (all from Tecomedical, Germany), CTX (Nordic Bioscience, DK)
Statistics: Kruskal-Wallis-Test followed by Mann-Whitney-Test and Bonferroni-Holm correction for multiple comparisons.

Results
All data were normalized to the control group (= 100%) to account for differences between the serials. The results of the ELISAs OPG, CICP, CTX and TRAP 5b were normalized to total protein and expressed in % to control. All results of the RANKL ELISA were under detection limit of the assay (lowest standard value) and could not be used for further analysis (data not shown).
Fusion assay
OPG was significantly reduced after stimulation with BMP-2 and IGF-I/TGF-β1. COL-I was also significantly reduced in the BMP-2 group, but under IGF-I/TGF-β1 stimulation the production was enhanced (Fig. 1a,b). A significant stimulating effect of all growth factors was seen on the TRAP activity at day 5. At day 15, however, TGF-β1 and the combination with IGF-I reduced significantly the number of fused osteoclast-like cells (Fig. 1c-h).
Resorption assay
The application of IGF-I, TGF-β1 and the combination thereof stimulated significantly the COL-I production of osteoblast-like cells (Fig. 2a). No effect on osteoclastic resorption activity was detected (Fig. 2b-g).

Discussion
The co-culture system is seldom used for cell culture studies but can give further information on the effect of factors resulting from interacting cells types. The assays used in the present study mainly focused on the development of monocytes to multinuclear osteoclast-like cells (fusion assay) and the resorption activity of these cells (resorption assay) influenced by the co-cultured osteoblast-like cells.

Looking at the influence of growth factors on osteoclast-like cells in co-culture, differentiation dependent effects were seen. Comparing the results to previous studies with single cell culture with identical experimental setups not many similarity could be found. The results were partially unexpected and also contrary to other published results of pure osteoblast or osteoclast culture experiments. This highlights the necessity of co-culture experiments to gain more information on the effect of drugs in the interaction of osteoblasts and osteoclasts.

References
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