DIFFERENTIATION BUT NOT PROLIFERATION OF OSTEOBLASTS IS STIMULATED BY IGF-I AND TGF-BETA1 IN VITRO

Wildemann, B; Schmidmaier, G; Lübbeustedt, M; Haas, N.P; Raschke, M
Dept. of Trauma and Reconstructive Surgery, Charité Virchow-Clinic, Humboldt-University, Berlin, Germany

Introduction
Growth factors are known to influence osteogenic and chondrogenic cells. In vitro and in vivo studies investigated the effect of various growth factors on different cell types, cellular processes and on bone formation. Previous studies revealed a stimulating effect of locally applied IGF-I and TGF-β1 from Poly(D,L-lactide) coated titanium implants on fracture healing. However, also the PDLLA coating seemed to effect healing processes (1). The purpose of the present study was to evaluate the effect IGF-I (5% w/w) and TGF-β1 (1% w/w) and the carrier PDLLA on osteoblasts in cell culture.

Material and Method
Growth factors and implants:
Recombinant human IGF-I (5% w/w, 30µg) and recombinant human TGF-β1 (1% w/w, 6µg) (R&D-Systems, USA) were incorporated into Poly(D,L-lactide) used for implant coating. Following samples were investigated:
1. titanium kirschner-wires uncoated
2. titanium k-wires coated with PDLLA
3. titanium k-wire coated with PDLLA and IGF-1 + TGF-β1

Cell culture: The osteoblast cell line hFOB 1.19 (10,000 each well) was used for the experiments and cultured in DMEM+HEPES at 34°C at 7% CO₂. The pH of the medium during the experiment was 7.6 to 8.0. The implants were added to the osteoblast culture and removed after 1, 12, 24h, 2, 4, and 10 days (n=6 each time point and each sample). Every second day the culture medium was changed. All cell cultures were incubated for in total 10 days. Control: hFOB 1.19 cell culture without wires

- Trypan blue stain was used to count live and dead cells (Proliferation/viability assay).
- Cell activity/metabolism was determined by the WST-test (Tetrazolium salt is converted by mitochondrial dehydrogenases to formazan).
- Collagen-1 synthesis was analyzed with the Procollagen-ELISA (Prolagen, Metra Biosystem, USA).

Immunological test: mice monocytes/macrophages (line J774 A.1) were incubated for 3 days with the cell culture medium from the degradation test (see above) and the IL-1β production was measured with an ELISA test.
Statistics: one way ANOVA and Dunnett Post Hoc test

Results
Cell culture
The vitality test showed no influence of the tested implants on the cells and ranged between 93 to 97% in all groups.

- **Proliferation:** The cell count revealed a decrease in the amount of cells after treatment with growth factors (group 3). After incubation with growth factor for 4 days or longer the reduction was significant. The PDLLA coating alone (group 2) caused only a slight decrease in the proliferation activity compared to the control culture. The titanium implant stimulated slightly the proliferation activity in osteoblasts (Figure 1).

- **Cell activity:** No differences were detectable in the activity of the osteoblasts after incubation with the titanium implant or the PDLLA implant compared to the control culture. The cells treated with growth factors showed a significantly reduced mitochondrial activity. However, when the culture was incubated for 4 and 10 days the cell activity increased in this group. Compared to the decreased amount of cells in each well, the result led to the conclusion that the activity is enhanced in the growth factor treated cells. (Figure 2)

- **Collagen-1 synthesis:** A significant increase in the collagen-1 production was detectable in the osteoblast culture treated with growth factors. Even after a 1h incubation of the cells with the growth factor coated implants an enhanced collagen-1 production was measured. The PDLLA coating had no effect on the collagen-1 production (Figure 3).

Discussion
The cell culture results clearly demonstrate that IGF-I and TGF-β1 incorporated into a polylactide coating influence osteoblast differentiation. The effect on the osteoblastic cell line was not the stimulation of cell proliferation but the stimulation of cell differentiation. No negative effect was seen in the vitality of the cells. Even an increase in cell activity was notable comparing the mitochondrial activity to the amount of cells in each well. In the growth factor group less cells were countable compared to the control culture, however the dehydrogenase activity increased at the later incubation time points (days 4 and 10). The effect of the PDLLA-coating on the osteoblasts was not very pronounced. No differences in proliferation activity, cell vitality and differentiation were detectable. Same results were found for the uncoated titanium implant. In conclusion no negative effect of the PDLLA on osteoblasts was found. The growth factors affect the osteoblasts more to differentiate than to proliferate. Neither the PDLLA-coating nor the incorporated growth factors evoked an immunological reaction in mice monocytes/macrophages. (1) Schmidmaier G, 2001 Bone 28:341-350.

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