P42-INFLUENCE OF TGF-ß1 AND CULTURING MEDIUM ON ALP EXPRESSION IN HUMAN PULP FIBROBLASTS WITH DIFFERENT ROOT DEVELOPMENT

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Key Words
Pulp fibroblasts, TGF-ß1, ALP, root development

Introduction
TGF-ß1 is one of the most important growth factors in human pulp fibroblast differentiation. The influence of TGF-ß1 is mediated by its concentration. Furthermore the regenerative potential of human pulp fibroblast depends on their origin. The aim of this study was to compare the expression of ALP in cultures of human pulp fibroblasts with different root development under the influence of various TGF-ß1 concentrations in vitro.

Materials and Methods
Human pulp fibroblasts gained from third molars, were separated in two groups according to their root development and cultured in D-MEM with 25mM Hepes (PAA), 10% FCS (Sigma) and 50μg/ml Gentamycin (Biochrom). After the third passage, cells were seeded to 25.000 in 24 well plates. A third of every culture was treated with D-MEM + 10% FCS, D-MEM + 0.1% FCS and D-MEM + ITS (Insulin-Selenit-Transferrin, BD Biosciences) respectively. Additionally the cultures were treated with 0.5; 1; 5 and 10ng/ml TGF-ß1 (R&D). ALP expression was estimated by the 4-NPP method over a period of 32 days. Statistical analysis was done by analysis of variance.

Results
A specific mode of ALP expression was observed according to the media supplied. From day 11 to day 32 the ALP expression in cultures from teeth with incomplete root development was significantly higher than in the other group ($p < 0.05$) independent of the culturing conditions. The onset in ALP expression was significantly different in both groups as well ($p < 0.05$). This was also not effected by the culturing conditions. TGF-ß1 had no significant influence on the ALP expression in each group, but the peak in ALP expression was recorded at a concentration of 0.5ng/ml compared to the control cells. Higher and lower concentrations diminished the ALP expression.

Discussion
With the completion of the root (closed apex) the mineralizing capacity of human pulp fibroblasts decreased. This clinical evidence was approved by the significant difference in ALP expression in both groups shown by a higher ALP expression from day 11 to 32 and by an earlier onset (day 4 vs. day 8) in cultures with incomplete root development. The substitution of TGF-ß1 in vitro or in vivo influenced the differentiation of human pulp fibroblasts into odontoblast like cells and the secretion of new dentin matrix. In this study TGF-ß1 at a concentration of 0.5ng/ml increased the ALP expression but not significantly, while every other concentration diminished it. So, a special concentration of growth factors like TGF-ß1 is a critical parameter for the characteristics of the cells in vitro.

Conclusion
The donor specific origin of the cells is more important for their regenerative potential and characteristics than the addition of growth factors like TGF-ß1. However a certain concentration of TGF-ß1 (0.5ng/ml) improved the mineralizing properties of human pulp fibroblasts in cultures of incomplete root development as well as in cultures of complete root development in vitro as measured via ALP expression.