

Human dental pulp fibroblasts release angiogenic growth factors

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INTRODUCTION: After pulp amputation, complete pulp healing requires not only reparative dentin production but also fibroblast proliferation, nerve fiber growth and neoangiogenesis. We hypothesized that angiogenic growth factors may be released from pulp fibroblasts and that their release may be necessary for complete pulp healing after injury.

METHODS: Human pulp fibroblasts were prepared from immature third molars. Cells were cultured in 35-mm-diameter culture dishes (Becton Dickinson Labware, Lincoln Park, NJ) in minimum essential medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 UI/ml penicillin, 100µm streptomycin and 0.25 µg/ml amphotericin B (Fungizone). Injuries to fibroblasts were performed mechanically with sterile scalpels to disrupt the fibroblast monolayer. The media obtained after a contact period of 5 hours, 1, 2 and 3 days with injured or intact cells were then used for the quantification of angiogenic factors.

The growth factors investigated were platelet-derived growth factor (PDGF-AB), basic fibroblast growth factor (FGF-2) and vascular endothelial growth factor (VEGF), and were assayed using the sandwich enzyme linked immunosorbent assay (Quantikine Cytokine ELISA kit, R&D system, France).

Each experiment was done in triplicate. Error bars reflect the standard deviation and probability values were assessed using the Mann-Whitney nonparametric test. $p < 0.05$ was considered significant.

RESULTS: Intact human pulp fibroblasts release angiogenic growth factors: PDGF-AB (169.69±21.89 pg/ml), FGF-2 (299.37±71.25 pg/ml) and VEGF (907.55±77.69 pg/ml). After injury, there was a significant increase in growth factor concentration (as compared to intact cells). This promotion was 150.59% for PDGF-AB; 23.35% for FGF-2 and 20.6% for VEGF. This increase was obtained 5 hours after injury and then returned to initial values. There was no significant

change in expression of these factors after 1 day, 2 days and 3 days (figure 1).

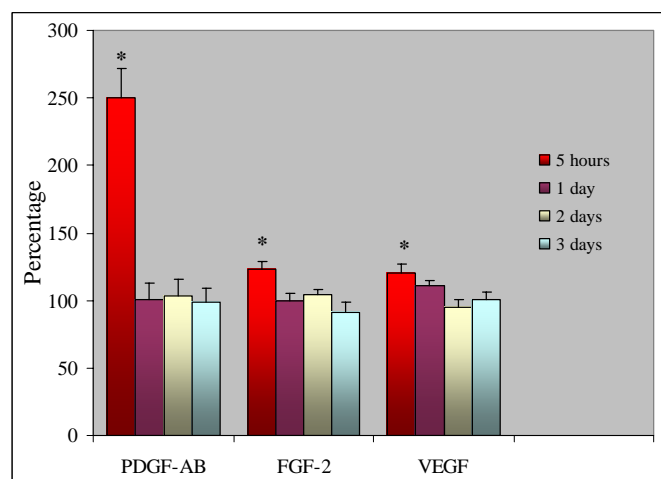


Fig. 1: Expression of angiogenic factors in dental pulp. PDGF-AB, FGF-2 and VEGF expression were up-regulated after 5 hours of injury and returned to initial values after 1, 2 and 3 days. This secretion was quantified by ELISA and was expressed as percentage of control (without injury). * Significant difference ($p < 0.05$).

DISCUSSION & CONCLUSIONS: Our work clearly demonstrates that human pulp fibroblasts secrete PDGF-AB, FGF-2 and VEGF particularly after injury. The highest concentration was that of VEGF (907.55±77.69 pg/ml). The release of these factors is very rapid and corresponds well to the pulp physiology. After pulp injury, the migration of odontoblast progenitor cells to the injury site may require newly formed blood vessels.

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