

## An immunohistochemical study of the human periodontal ligament during sperimental orthodontic movement

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Orthodontic tooth movement is characterized by remodeling changes in dental and parodontal tissues, including dental pulp, periodontal ligament (PDL), alveolar bone, and gingiva. These tissues, when exposed to varying degrees of magnitude, frequency, and duration of mechanical loading, express macroscopic and microscopic changes. The different modification of periodontal ligament during load deformation can be monitored by analysis of the expression of different collagen types, fibronectin and vascular endothelial growth factor. The aim of this study was to evaluate PDL collagen types I and IV, fibronectin and vascular endothelial growth factor (VEGF) modification induced by application of a precalibrated and constant orthodontic strength at different stages of treatment. For the study we utilized a coil spring NiTi 50 gr. and in vivo samples of 20 maxillaries and mandibular premolars of patients aged from 13 to 18 years subject to orthodontic treatment. These teeth were extracted at 1, 7, 14, 21 and 28 days from application of force respectively. The extraction of the PDL was effected by scarifying the radicular surface on the pressure and tension side. The results were compared with periodontal ligament samples of the normal homologous teeth (control). The periodontal ligament samples were fixed in 3% paraformaldehyde in a 0,2M phosphate buffer at pH 7,4. The following primary antibodies were used: anti collagen I, anti collagen IV, anti fibronectin and mouse monoclonal anti-VEGF. Sections were then observed and photographed using Zeiss LSM 510 confocal microscope. We analyzed fluorescence intensity and compared with the control side. The signal of type I collagen is negative in tension and pressure side after 1 day, showed an increased respect to control, in the tension and pressure side, until 7, 14 and 21 days. After this stage in both sides maintained the same values of the control. The immunofluorescence of type IV collagen is negative, in both sides, after 1 and 7 days. At 14, 21 and 28 days from treatment, increased gradually in pressure side and maintained the same values of the control in tension side. The observation of fibronectin showed strongly immunofluorescence at all stages of treatment. After 1, 7 and 14 days the immunofluorescence of VEGF is negative in pressure side, and positive in tension side. In the last observation periods at 21 and 28 days, VEGF signal showing, in both sides, significant increase when compared with the control group. These findings suggest that: the increase of collagen type I and fibronectin could indicate that the solicitation by orthodontic force could determinate an increase of metabolic activity in the periodontal ligament. The rapid modification after the start of tooth movement, suggest that VEGF may be involved in the early stages of periodontal remodeling during orthodontic tooth movement, when occur rapid changes in local blood circulation. The initial decrease of collagen type IV, indicate a loss of vascular component in the early stages of movement in fact this protein is localized in perivessel zones.