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Biochemical and immunohistochemical analysis of tissue inhibitor of metalloproteinases-1 (TIMP-1) in human sound dentin

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Introduction. Matrix metalloproteases (MMPs) are a family of enzymes mainly produced by cells of connective tissue as proenzymes and secreted in the extracellular matrix. MMPs are calcium/zinc-dependent proteinases and operate a specific proteolytic activity on most constituents of the extracellular matrix. The effects of MMPs are regulated by tissue inhibitor of metalloproteinases (TIMPs), small regulatory proteins that can bind ubiquitously different enzyme forms. Different studies showed that MMPs are also involved in dentinogenesis. Additionally it has been shown that some enzymes such as MMP-2 and MMP-9 are involved in autodegenerative processes, such as the degradation of dentin matrix in caries lesions.

Purpose. The purpose of this study was to identify different enzyme isoforms in association with TIMP-1, through co-immunoprecipitation/immunoblotting and the evaluation of enzyme localization by immunohistochemical techniques and light and electron microscopy analysis.

Methods. Proteins were extracted from human dentin powder previously demineralized with 1%H3PO4 for 10min. Extraction buffer is composed of 5mM CaCl2, 100mM NaCl, 0.1% Triton X-100, 0.1% NONIDET (non ionic detergent), 0.1mM ZnCl2, 0.02% NaN3 and protease inhibitors in 50mM Tris-HCl pH6. The extracted proteins were subjected to co-immunoprecipitation and subsequent immunoblotting against MMPs and TIMP-1. Additional samples were prepared to analyze the presence of TIMP-1 in light microscopy using diaminobenzidine colorimetric assay. The evaluation of TIMP-1 localization within the fine dentinal structure was performed also by immunogold labeling in FEI-SEM microscopy (Field Emission In lens Scanning Electron Microscopy).

Results. Co-immunoprecipitation/immunoblotting analysis show the association TIMP1/MMP-2 and TIMP1/MMP-9 in human sound dentin. Light and electron microscopy revealed a strong presence of TIMP-1 in intratubular dentin rather than in the intertubular. Their association with MMPs is finely adjusted and an imbalance could lead to caries pathology and failure of adhesive systems.

Keywords: biochemical analysis, immunohistochemical analysis, MMPs, TIMPs, light microscopy, electron microscopy