

Biochemical and immunohistochemical identification of MMP-3 in human sound dentin

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Objective Matrix metalloproteinases (MMPs) are a family of Zn- and Ca-dependent enzymes, collectively called matrixins. MMPs are important components in many biological and pathological processes because of their ability to degrade all extracellular matrix (ECM) components. Within the human dentin matrix, the function of endogenous MMP-3 and its distribution is unclear. Thus, the aim of the present study was to evaluate the presence and distribution of MMP-3 within human sound dentin by biochemical and immunohistochemical methods.

Methods Powdered dentin from extracted human teeth was prepared and partially demineralized with 1% H₃PO₄ for 10 min. Untreated powder was used as control. The presence of MMP-3 in mineralized and partially demineralized dentin powder was assessed using a colorimetric assay system that allows direct measuring of MMP-3 levels (Quantisir™, Epigentek, USA). All experiments were run in triplicate (N=3). To immunohistochemically identify the distribution of MMP-3 within the dentin organic matrix, a FEI-SEM analyses, was performed on cryo-fractured dentin fragments using a monoclonal antibody anti-MMP-3.

Results The amount of MMP-3 in control samples was approximately 3.12 ng/μL, while it significantly decreased in partially demineralized dentin (2.452 ng/μL). The FEI-SEM analysis also revealed positive immunolabeling patterns for MMP-3. The presence of MMP-3 was predominantly localized on the intertubular collagen fibrillar network with the MMP-3 directly or indirectly bound to the collagen fibrils.

Conclusions As a step toward understanding the role of MMPs in human dentin biology and pathology, our study clearly identifies both the presence and the distribution patterns of MMP-3 in human sound dentin by means of immunohistochemical and biochemical techniques. Nevertheless, further investigations are required to clarify the possible involvement of MMP-3 in pathological condition of the dentin-pulp complex.

Key words

MMPs, MMP-3, dentin matrix