

Identification of MMP-8 in human sound dentin

Annalisa Mazzoni¹, Veronica Papa², Pietro Gobbi³, Gabriella Teti¹, Lorenzo Breschi⁴, Giovanni Mazzotti¹

¹ Department of SAU & FAL, University of Bologna, Italy

² Department of Sport, Sciences & Health, University of Cassino, Italy

³ Department of SUNT, University of Urbino, Italy

⁴ Department of Biomedicine, University of Trieste, Italy

Objective Matrix metalloproteinases (MMPs), a class of Ca/Zn-dependent endopeptidases, are secreted by odontoblasts as proenzymes that remain trapped within the mineralized dentin matrix. Activation of these proenzymes is a critical step that leads to dentin extracellular matrix breakdown. The study aimed to assess presence, localization and distribution of MMP-8 in human sound dentin by biochemical and immunohistochemical assays.

Methods The presence of MMP-8 in human sound dentin was assayed using a colorimetric assay system that allows direct measuring of MMP-8 levels (QuantisirTM, Epigentek, USA): powdered dentin prepared from extracted human teeth was prepared and 1) partially demineralized with 1% H₃PO₄ for 10 min or 2) untreated (control). To further detect the localization and distribution of MMP-8 within the dentin organic matrix, dentin cryo-fractured fragments were obtained from human sound teeth, partially decalcified in 0.5 M EDTA pH 7.4 for 30min and submitted to a pre-embedding immunolabeling technique, using primary monoclonal antibodies anti-MMP-8 and exposed to a secondary antibody conjugated with gold nano-particles. Observation was performed under FEI-SEM.

Results In mineralized dentin the amount of MMP-8 was 3.32 ng/μL, while the MMP-8 level in the partially demineralized dentin powder significantly decreased (2.45 ng/μL) compared to levels obtained in mineralized dentin. FEI-SEM analyses confirmed that MMP-8 is an endogenous component of the human dentin organic matrix. The labelling pattern of the gold particles bound to the antibody anti-MMP-8 was clearly detectable in the dentin fragments as spherical, highly electron reflective spots localised in the peritubular dentin, revealing the three-dimensional relationship between these proteinase and the collagen fibrils.

Conclusion The role and function of dentin MMPs is not well known, but they have shown to contribute to auto-degenerative processes in dentin, such as inflammation of dental pulp, progression of caries lesions and, more recently, degradation of dentin matrix exposed during dental bonding procedures. This study demonstrated that MMP-8 is an intrinsic constituent of the fibrillar network of the human dentin organic matrix, thus supporting that this enzyme may play important roles in the degradation of the dentin organic matrix.

Key words

MMPs, MMP-8, human dentin