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Different enzyme extraction methods for human 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. 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 auto-degenerative processes, such as the degradation of dentin matrix exposed during the dentin bonding procedures.

Objective The purpose of this study was to analyze different enzymes extraction methods in relation to the assay to be performed, i.e. zymography and western blotting (WB) for MMP-2 and -9.

Methods Proteins were extracted from human dentin powder and demineralized with 1% phosphoric acid for 10min. Two different extraction buffers with different extraction ability were tested. Buffer A: 7M urea, 2M thiourea, 4%CHAPS in 20mM Tris-HCl pH7.4. Buffer B: 5mM $CaCl_2$, 100mM NaCl, 0.1% Triton X-100, 0.1% NONIDET, 0.1mM $ZnCl_2$, 0.02% NaN_3 and protease inhibitors in 50mM Tris-HCl pH 6. Bradford assay was performed to quantify concentration and MMP-2 and -9 were analyzed with two different biochemical techniques: WB and zymography.

Results The protein quantity extracted with buffer B was 10-fold less than buffer A. WB analysis of dentin protein extracted with buffer A showed presence of both MMP-2 and -9, while no gelatinolytic activity was found in zymography. Conversely, after the use of buffer B, despite a low rate of protein detection in WB, extracted enzymes showed intense gelatinolytic activity.

Conclusion To preserve the enzymatic activity, enzymes must retain their native form. Buffer A has strong reducing properties with high extraction ability, while buffer B is a non-reducing agent with lower extraction ability. The results of this study showed that despite lower extraction capability, buffer B allows to retain enzymatic activity of MMP-2 and -9 in human dentin.

Key words
MMP-2, MMP-9, dentin, Western Blotting, Zymography