The contribution of matrix metalloproteinase in enzymatic degradation studies in dental bonding

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ABSTRACT

Introduction: Matrix metalloproteinases (MMPs), also known as matrixins, are calcium-dependent zinc-containing endopeptidases. Phosphoric acid in the etch-and-rinse bonding process and acid primers in the self-etch process are implicated in the release of these proteases, and their activation by several non-collagen proteins also released from dentin by the etching. The Most MMPs are synthesized and released from the odontoblasts in the form of proenzymes, requiring activation to degrade extracellular matrix components. Objective: The objective of this study is to assess and determine the contribution of MMP in the enzymatic degradation in the dental bonding. Materials and Methods: About six patients were divided into two groups: One group with a diagnosis of periodontitis and one control group which classified as being “healthy.” The MMP’s assay and protein expression were carried out. The blood and saliva were collected from them and used for the further analysis. Result: This study is done to evaluate the role of MMPs to enzymatic degradation at dental bonding. The release and subsequent activation of these endogenous enzymes during the dental restorative procedures are believed to be responsible of the dentin–adhesive bonding failure. Conclusion: From this study, it is so evident that the release of MMPs plays a key role in the failure of the dental restorative procedures.

KEY WORDS: Dentin bonding, Enzymatic degradation, Hybrid bond layer, Matrix metalloproteinases

INTRODUCTION

Matrix metalloproteinases (MMPs) are a family of nine or more highly homologous Zn++ - endopeptidases.[¹] They are a family of host-derived proteolytic enzymes that are usually trapped in the mineralized dentin matrix. They can carry out the hydrolyzation of the organic matrix of demineralized dentin. After the resins bond to the dentin, there is slight exposure of the collagen fibrils at the bottom of the hybrid layer owing to imperfect resin impregnation matrix. Exposed collagen fibrils can be affected by MMPs inducing hydrolytic degradation, which might result in a reduction in the bond strength of the restoration. The dentin MMPs are usually synthesized by the odontoblasts as proenzymes. The MMPs can be activated by self-etch and etch-and-rinse adhesives which can lead to the failure of the restorative procedures.[²] Enzymatic degradation in the exposed collagen matrix, due to the various host-derived enzymes, is the main reason for the bonded interface destruction. Recently, several matrix proteins such as several MMPs and cysteine cathepsins are identified in the dentin and are proposed to be responsible for the exposed collagen fibrils digestion.

Relevant studies on MMPs have led to the various tentative efforts to prevent such enzymatic degradation, and thereby, to increase the shelf-life of the dental restorations and to prevent the action of the extracellular matrix proteins.[³]

The Role of Dentin MMPs

The relationship between the active MMPs and the carious lesions provide a background for the recent advances in the research regarding the effects of MMPs on the stability of the dentin hybrid layer bond. The various MMPs observed in the carious lesions includes MMP-2 (gelatinase), MMP-8 (collagenase), MMP9 (gelatinase), and MMP-20 (enamelysin). The dentin protein matrix consists of 90 % Type I collagen and 10% of non-collagenous proteins.

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proteins. The collagen fibrils can be degraded by MMP 8, MMP 2, and MMP 9. This is usually carried out after the demineralization of acid in the dentin which is in the carious lesion. The various MMPs are either present in the saliva, pulp or present within the dentin matrix which gets released during the destruction of caries in the dentin. The MMPs are seen in higher concentration and extensively along the enamel-dentin junction and also at the pre-dentin. The increased presence of MMPs can contribute to the caries widening along the dentinoenamel junction and eventually progresses into the dentin. The MMPs are released by both organic and inorganic acids. They are activated by the other proteins and organic acids in the oral environment or due to the bonding adhesives. The fibril exposure occurs due to the nano leakage. As time passes, there is bond degradation as a result of which there is loss of retention clinically. The retention of the restoration decreases by 20% in 5 years or less. The reduction in retention can lead to marginal staining and increased tooth sensitivity. This will ultimately result in the replacement of the restoration. Due to the increased enzymatic degradation and also due to the reduction in the retention of the restoration, there is increased nano leakage, marginal staining, and also chipping of the marginal ridge is also most likely to happen. Every change is basically based on the bond strength degradation that occurs over time.\textsuperscript{[4,8]}

\textbf{Application of MMPs}

The MMPs have always been considered the “bulldozers” as they destroy the extracellular matrix (ECM) which actually permits normal remodeling and also help in the destruction of the pathological tissues and also the invasion of tumor cells. Perhaps, the MMPs are now viewed to be playing sophisticated roles as the pruning shear that will help in the modulation and the regulation of the normal cellular behavior, cell-to-cell communication, and also in the progression of the tumor. This conclusion has further been confirmed by the recent identification of specific matrix and non-specific matrix substrates for MMPs.\textsuperscript{[9]} The abnormality in the connective tissue degradation in various orthopedic joint diseases such as rheumatoid arthritis and osteoarthritis is also most likely due to the imbalance in the expression of the MMPs. MMPs has also been observed and detected in the ligament, tendon, and cartilaginous tissues of the joint. The role of MMPs have now been discovered to involve in the maintenance of healthy tissue development, remodeling, cell growth, migration, differentiation, and apoptosis because of their ability to cleave a wide range of extracellular matrix substances. In drug therapy, the regulation of MMPs which targets for inhibition in multiple steps is very much useful.\textsuperscript{[10]} The MMPs helps not only in the degradation of the extracellular matrix but also helps a lot in the process of angiogenesis. They help to detach the pericytes from the vessels which are already undergoing angiogenesis, by releasing the ECM-bound angiogenic growth factors, and thereby, the exposure of cryptic proangiogenic integrin binding sites in the extracellular matrix component fragments and also aid in the cleavage of endothelial cell–cell adhesions.\textsuperscript{[11]} There is also negative contribution of the MMPs by the generation of the endogenous angiogenesis inhibitors to the angiogenesis by the proteolytic cleavage of the chains of collagen fibers and plasminogen and also modulates cell receptor signaling by the cleavage of the ligand-binding domains.\textsuperscript{[12]}

\section*{MATERIALS AND METHODS}

Two groups comprising six patients in total was chosen. One group of patients with the diagnosis of “periodontitis” and the other group was taken as the control group which is classified as being “healthy.” The blood and the saliva were collected from the two groups of patients for further analysis. The MMP’s assay and the protein expression assay were carried out.

\textbf{Assay of MMPs}

Saliva concentrations of MMP-13 were determined by enzyme-linked immunosorbent assay using ELISA kits (Boster biological technology, USA). Determination of MMP-13 levels was carried out according to the manufacturer’s instruction.

Add Standard working solution of different concentrations to the first two columns: Each concentration of the solution is added into two wells side by side (100 µL for each well). Add samples to other wells (100 µL for each well). Cover the plate with sealer provided in the kit. Incubate for 90 min at 37°C. Remove the liquid of each well, do not wash. Immediately, add 100 µL of Biotinylated Detection Ab working solution to each well. Cover with the Plate sealer. Gently mix up. Incubate for 1 h at 37°C. Aspirate or decant the solution from each well, add 350 µL of wash buffer to each well. Soak for 1–2 min and aspirate or decant the solution from each well and put it dry against clean absorbent paper. Add 100 µL of horseradish peroxidase conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C. Aspirate or decant the solution from each well, repeat the wash process for 5 times as conducted in step Add 100 µL of Substrate Mixture Solution to each well. Cover with a new plate sealer. Incubate for not more than 5 min at 37°C. Protect the plate from light.

Determine the intensity value of each well at once after the substrate reaction time.
RESULTS AND DISCUSSION

Results are expressed as a mean ± standard error of the mean, \( n = 3; ^* P < 0.001 \), statistically significant as compared with healthy control.

The results revealed that the healthy control seemed to show less concentration of absorption [Figure 1] when compared with the periodontitis control teeth. In addition, the \( p \) value being <0.001, which is statistically significant, there is a vast difference in the colorimetric values which is suggestive of the release of MMPs, which plays a major role in the digestion of the extracellular proteins and leading to the failure of restorations and also the bonding to the dentin.

As shown in Figure 2, saliva shows increased expression of MMP-2 (lane 1) as compared with healthy control (Lane 2). The MMP’s assay clearly shows that the periodontally compromised tooth seems to have increased release of MMPs, due to which there is marginal leakage, and nano leakage after the restoration done, due to which the digestion of the extracellular proteins and reduction in the bond strength also occurs. The scope of MMPs in the field of biotechnology has improved so well, because it’s been used as a biomarker in oncology. Being a diagnostic and a prognostic tool, MMPs are promising and efficient biomarkers.[13] It is also observed that the levels of MMP-9 are elevated in the breast cancer patients.[14-16]

CONCLUSION

The MMPs have conflictory roles in angiogenesis and the increased levels or expressions of which acts as an efficient biomarker. MMPs inhibitors are designed and are under clinical trials to prevent the failure of the restoration and also to increase the quality of restoration. The expression of MMPs is used as diagnostic tools in various tumors such as breast cancer and ovarian cancer. The MMP’s assays serve as an sample tool to diagnose and also the accuracy is pretty statistically significant compared to other assays. Thus, by exploring the role of various extracellular components and their composition, we can come up with diagnostic aids which are more accurate than the conventional assays and can be used for the early detection of cancers, being the biomarker to be expressed initially in case of a tumor. Increased secretion of MMPs in case of gingivitis, periodontitis is one of the main reasons for the reduction in the MMPs, and MMPs inhibitors are used for a better restoration.[22]

REFERENCES


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