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Identification TIMP-1 and TIMP-2 in Human Radicular Dentine - In Vitro Study

Kandaswamy Eswar[1], Rubin Mohamed Ismail[2], Hannah Rosaline[3], Nagendrababu Venkateshbabu[4], Deivanayagam Kandaswamy[5]

[1]Intern; [2]Former Postgraduate Student; [3]Additional Professor; [4]Reader; [5]Professor and Head, Department of Conservative Dentistry and Endodontics, Faculty of Dental Sciences, Sri Ramachandra University, India; frozenfire.eswar@gmail.com

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Abstract

Aim: To identify TIMP – 1 and TIMP – 2 in human radicular dentine using confocal laser scanning microscopy. **Materials and Methods:** Thirty freshly extracted non carious human single rooted pre molars were obtained and stored in isotonic saline at -70°C prior to use. All the teeth were decoronated at the CEJ using a diamond. Teeth were divided into 2 groups (Group 1: TIMP-1 analysis n = 15; Group 2: TIMP-2 analysis n = 15). Teeth were sectioned using a hard tissue microtome, mounted and viewed under confocal laser scanning microscopy. **Results:** TIMP-1 and TIMP-2 were detected in radicular dentine and were seen to be distributed more towards the inner dentine layer closer to the pulp. **Conclusion:** Due to a shorter half life of TIMP-1 and 2 as compared to the MMP, there is a need to use MMP inhibitors prior to obturation of the root canal.

Keywords: Bond Degradation, Collagenolysis, Confocal Laser Scanning Microscopy, Matrix Metalloproteinase, Radicular Dentine, Tissue Inhibitors of Metalloproteinase

1. Introduction

Matricins, a collective terminology for Matrix Metalloproteinases (MMPs), are a class of endopeptidase that degrades native and denatured collagen and also other Extracellular Matrix molecules including collagen (ECM) (Bikerdal-Hansen 1993, Kreis 1999, Visse 2003). 23 MMPs have been cloned in humans (Visse 2003, Nagase 1999, Nuttall 2004). A fundamental role in oral tissue development and remodeling is played by these MMPs. They also remodel the organic matrix of dentin and get incorporated into mineralized dentin (Hall 1993, Martin 2000, Sulkala 2002). MMP – 8 (collagenase), MMP – 2 and 9 (gelatinases), MMP – 3 (stromelysin), MMP – 20 (enamelysin) have been localized in human dentin (Sulkala 2003, Martin 2000, Mazzoni 2006, 2007, 2009, 2011a, 2011b, Sulkala 2002). Recently, these MMPs have been linked to loss of adhesion of composite restorations with passage of time (Carrilho 2005, Hebling 2005). Collagen fibrils restored by adhesive systems undergo degradation of the exposed collagen fibrils by these Matrix Metalloproteinases. (Carrilho 2007 a,b; Breschi 2008). The MMPs are counteracted by a natural defense mechanism in the form of Tissue Inhibitors of Metalloproteinases (TIMPs) (Bikerdal-Hansen 1993).

4 types of TIMPs have been identified and characterised in the human body. They are secreted proteins having a low-molecular weight. Their major action is to bind to MMP in an equimolar ratio and inhibit them (Visse

2003). TIMP-1, TIMP-2, and TIMP-4 are usually found in associated with membrane-bound proteins (Yu 2000).

Tissues remodeling is largely controlled by both Matrix Metalloproteinases (MMPs) and the Tissue Inhibitors of Metalloproteinases (TIMPs). However, any disturbance of the balance of MMPs and TIMPs will result in pathological conditions, including rheumatoid and osteoarthritis, cancer progression, cardiovascular diseases and dental caries (Joost 2006, Becher 2008).

Ishiguro et al. in 1994 concluded that TIMP-1 concentration in radicular dentine was higher than that in the cementum. Later, the study done by Leonardi & Loreto in 2010 provided evidence for increased TIMP-1 immunolabelling in carious teeth. However, no study has localized the presence of TIMP-2 in radicular dentin and the effect of dentine removal in the form of root canal cleaning and shaping procedures on the removal of these TIMPs.

Hence the aim of the study was to study the distribution of TIMP-1 and 2 in normal radicular dentine.

2. Materials and Methods

2.1 Extraction of Sample

Thirty freshly extracted non carious human single rooted pre molars were obtained after informed consent from the Department of Oral and Maxillofacial Surgery at Sri Ramachandra University. The protocol to collect samples from human donors was approved by the Ethics Committee of Sri Ramachandra University. The teeth were stored in isotonic saline at -70°C prior to use. All the teeth were decoronated at the CEJ using a diamond disc under cold saline spray and the root portion was used for further analysis. Teeth were divided into 2 groups (Group 1: TIMP-1 analysis n=15; Group 2: TIMP-2 analysis n=15). Prior to confocal laser scanning microscopic analysis the teeth were sectioned using a hard tissue microtome at mid root level 4 mm below the CEJ. The thicknesses of the sections were 400µm.

2.2 Immunohistochemistry

The tooth sections were fixed with fixative agent containing 4% paraformaldehyde and 0.1% glutaraldehyde buffered with 0.1 M sodium cacodylate, at pH 7.2 and mounted onto a glass slide. The slides were washed for 5 min in Phosphate Buffered Saline (PBS) containing 0.1% Triton X with gentle agitation. The sections were incubated with the following primary antibodies. For the localization of TIMP-1, rabbit polyclonal anti-TIMP-1 (Abcam, UK) was used. For TIMP-2, Rabbit polyclonal anti TIMP-2 (Abcam, UK) was used. The antibodies were diluted to a concentration of 1:50 with PBS that contained 0.1% Triton X and 1% Bovine Serum Albumin (BSA) and incubated overnight at 4°C. The sections were washed twice in PBS containing 0.1% Triton X and 1% Bovine Serum Albumin (BSA) (Antibody staining buffer) each for 5 minutes to remove unbound primary antibodies. Goat polyclonal secondary antibody to Rabbit IgG coupled to FITC (Abcam, UK) was diluted to a concentration of 1:100 with PBS that contained 0.1% Triton X and 1% Bovine Serum Albumin (BSA). The tooth samples were incubated at room temperature for 90min. The sections were washed twice PBS to remove any unbound secondary antibody. Negative control sections were processed identically to the experimental slides except that they were incubated with secondary antibody and PBS instead of the primary antibody

The sections were then observed under a CLSM 510 META confocal laser scanning microscope (Zeiss, Jena, Germany) at 10X magnifications and at an excitation wavelength of 488 nm. The images were analyzed by two investigators independently.

3. Results

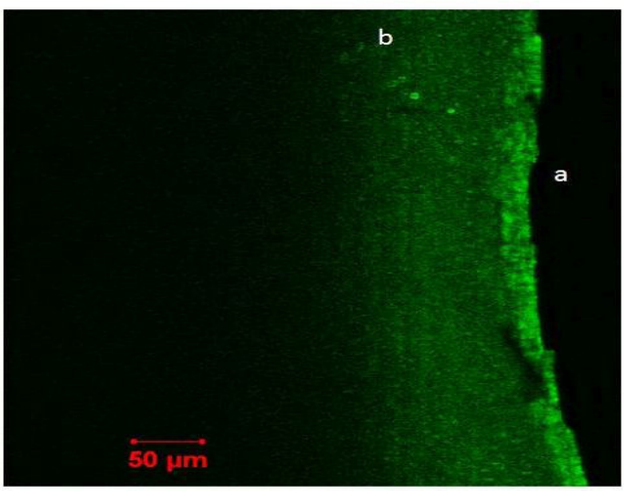

Figure 1. TIMP-1 in the normal radicular dentine.

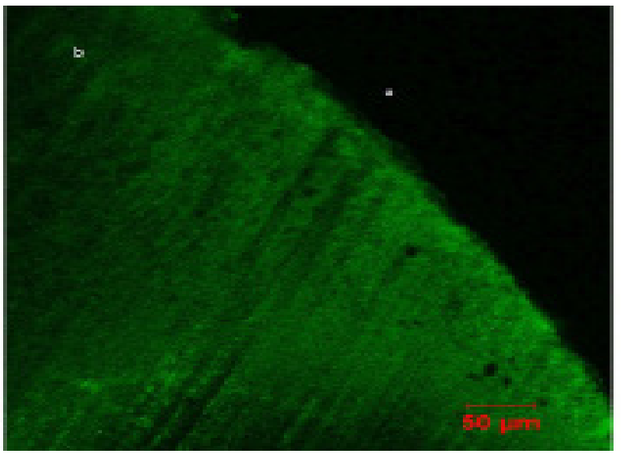

Figure 2. TIMP-2 in the normal radicular dentine.-

Confocal laser scanning microscopy revealed presence of TIMP-1 and TIMP-2 in the normal radicular dentine in all the samples tested. The TIMPs were found to be more concentrated towards the inner dentin layer close to the pulp.

4. Discussion

Dentin matrix contains both organic and inorganic components. The organic component is primarily composed of collagen matrix. The main collagen present in dentin is type I collagen (Goldberg 2004). Type III collagen has been detected in reparative dentine (Karjalainen 1986). Process of dentin matrix formation and remodeling is controlled by various active extracellular enzymes. These enzymes belong to a family of proteinases called the matrix metalloproteinases (Tjaderhane 2001).

MMPs are a family of zinc dependant endopeptidases (Woessner 1991). They are secreted in an inactive zymogen called pro-MMP. Pro-MMPs undergo activation before they degrade the extracellular matrix (Geiger 1981). The activation and degradation of MMPs are regulated by many natural processes and synthetic compounds (Visse 2003). MMPs are inhibited by Tissue Inhibitors of Metalloproteinases (TIMP).

TIMPs are secreted proteins. Goldberg et al. (2003) proved the presence of TIMP-1 and 2 in ameloblasts, odontoblasts and forming parts of rat incisor. Their study proved that TIMP-1 and 2 was lowest in the mantle dentin region where MMP-2 and 9 were particularly high. MMP–2 and 9 which come under the family of gelatinases are involved in dentin matrix destruction during carious process. These MMPs are activated at low pH (4.5) and destroy the collagen matrix following an increase in pH by buffering (Tjardahne 1998). The matrix metalloproteinases especially MMP-2 and 9 are present in both the radicular and coronal deep dentine and is thought to be responsible for a loss of bond strength of restorative materials and post endodontic restorations (Santos 2009, Boff 2007, Hisaishi 2010, Leitune 2010). The natural protease inhibitors present in dentin are the TIMPs which inhibit both MMP–2 and 9.

Our study evaluated the presence of TIMP-1 and 2 in normal radicular dentin. The results showed that TIMPs are present in the radicular dentin, more towards the pulp as the pulp is a know source of TIMP (Palosaari 2003). A study by Sa et al. (2011) has shown that TIMPs possess a lower half life than MMPs. Thus a reduction in TIMP after cleaning and shaping and their lower half life can cause lysis of the dentin collagen matrix by the remaining MMP in the radicular dentin. This is similar to studies where the MMPs that are present in the remaining dentine were activated by various root canal sealers (Huang 2008) and adhesive systems that are used to cement post endodontic restorations (Tay 2006) and caused a reduction in the bond strength over time. Chlorhexidine, a root canal irrigant is one of the synthetic substances known to possess strong MMP inhibitory activity even at low concentrations (Tjaderhane 1998). The disadvantage of using chlorhexidine as an irrigant is that it leads to the production of a potential carcinogenic substance when it reacts with sodium hypochlorite (para-chloroaniline) (James 2011). Several natural substances have been shown to have MMP inhibitory activity such as green tea and avocado (Demeule 2000; Garbisa 2001; Sartor 2002, Kut 1998, Huet 2004). Further research is required on these natural inhibitors before they can be used in the root canal to inhibit the MMPs leading to long term success of post endodontic restorations and obturation materials.

5. Conclusion

The TIMP-1 and 2 identified in the root canal can inhibit the MMP. However, due to a shorter half life of TIMPs and the ability of root canal sealers and adhesive systems to activate MMPs, it maybe necessary to use extrinsic natural or synthetic MMP inhibitors to ensure long term success of obturation and post endodontic restorations.

6. References

1. Becher N, Hein M, Uldbjerg N, et al. Balance between Matrix Metalloproteinases (MMP) and Tissue Inhibitors of Metalloproteinases (TIMP) in the cervical mucus plug estimated by determination of free non-complexed TIMP. Reproductive Biology and Endocrinology. 2008; 6:223–32.
2. Birkedal-Hansen H. Role of matrix metalloproteinases in human periodontal diseases. Journal of Periodontology. 1993; 64:474–84.
3. Boff LL, Grossi ML, Prates LH, et al. Effect of the activation mode of post adhesive cementation on push-out bond strength to root canal dentin. Quintessence International. 2007; 38:387–94.
4. Breschi L, Mazzoni A, Ruggeri A, et al. Dental adhesion review: aging and stability of the bonded interface. Dental Materials. 2008; 24:90–101.
5. Carrilho MR, Carvalho RM, de Goes MF, et al. Chlorhexidine preserves dentin bond in vitro. Journal of Dental Research. 2007; 86:90–4.
6. Carrilho MR, Geraldeli S, Tay F, et al. In vivo preservation of the hybrid layer by chlorhexidine. Journal of Dental Research. 2007; 86:529–33.
7. Carrilho MR, Tay FR, Pashley DH, et al. Mechanical stability of resin-dentin bond components. Dental Materials. 2005; 21:232–41.
8. Demeule M, Brossard M, Pagé M, et al. Matrix metalloproteinase inhibition by green tea catechins. Biochimica et Biophysica Acta. 2000 Mar 16; 1478(1):51–60.
9. Garbisa S, Sartor L, Biggin S, et al. Tumor gelatinases and invasion inhibited by the green tea flavanol epigallocatechin-3-gallate. Cancer. 2001; 91:822–32.
10. Geiger SB, Harper E. The inhibition of human gingival collagenase by an inhibitor extracted from human teeth. Journal of Periodontal Research. 1981; 16:8–12.
11. Goldberg M, Smith AJ. Cells and extracellular matrixes of dentin and pulp: a biological basis for repair and tissue engineering. Critical Reviews in Oral Biology & Medicine. 2004; 15:13–27.
12. Goldberg M, Septier D, Bourd K, et al. Immunohistochemical localization of MMP-2, MMP-9, TIMP-1, and TIMP-2 in the forming rat incisor. Connective Tissue Research. 2003; 44:143–53.
13. Hall R, Septier D, Embery G, et al. Stromelysin-1 (MMP-3) in forming enamel and predentine in rat incisor-coordinated distribution with proteoglycans suggests a functional role. Histochemical Journal. 1999; 31:761–70.
14. Hebling J, Pashley DH, Tjäderhane L. Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. Journal of Dental Research. 2005; 84:741–6.
15. Hiraishi N, Yiu CK, King NM, et al. Effect of chlorhexidine incorporation into a self-etching primer on dentine bond strength of a luting cement. Journal of Dentistry. 2010; 38:496–502.
16. Huang FM, Yang SF, Chang YC. Up-regulation of gelatinases and tissue type plasminogen activator by root canal sealers in human osteoblastic cells. Journal of Endodontics. 2008; 34:291–4.
17. Huet E, Cauchard JH, Berton A, et al. Inhibition of plasmin-mediated prostromelysin-1 activation by interaction of long chain unsaturated fatty acids with kringle 5. Biochemical Pharmacology. 2004; 67:643–54.
18. Ishiguro K, Yamashita K, Nakagaki H, et al. Identification of tissue inhibitor of metalloproteinases-1 (TIMP-1) in human teeth and its distribution in cementum and dentine. Archives of Oral Biology. 1994; 39:345–9.
19. Nowicki JB, Daniel S. An In Vitro Spectroscopic Analysis to Determine the Chemical Composition of the Precipitate Formed by Mixing Sodium Hypochlorite and Chlorhexidine. Journal of Endodontics. 2011; 37:983–8.
20. Karjalainen S, Söderling E, Pelliniemi L, et al. Immunohistochemical localization of types I and III collagen and fibronectin in the dentine of carious human teeth. Archives of Oral Biology. 1986; 31:801–6.
21. Kreis T, Vale R. Matrix metalloproteinases. In: Sternlicht MD, Werb Z, editors. Guidebook to extracellular matrix, anchor, and adhesion proteins, 2nd edn. San Francisco: Oxford University Press; 1999. p. 519–42.
22. Kut C, Assoumou A, Dridi M, et al. Morphometric analysis of human gingival elastic fibres degradation by human leukocyte elastase protective effect of avocado and soybean unsaponifiables (ASU). Pathologie Biologie (Paris). 1998; 46:571–6.
23. Leitune VC, Collares FM, Werner Samuel SM, Influence of chlorhexidine application at longitudinal push-out bond strength of fiber posts. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2010; 110:77–81.
24. Leonardi R, Loreto C, Immunohistochemical localization of tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) in human carious dentine. Acta Histochemica. 2010; 112:298–302.
25. Martin-De Las Heras S, Valenzuela A, Overall CM. The matrix metalloproteinase gelatinase A in human dentine. Archives of Oral Biology. 2000; 45:757–65.
26. Mazzoni A, Carrilho M, Papa V, et al. MMP-2 assay within the hybrid layer created by a two-step etch-and-rinse adhesive: biochemical and immunohistochemical analysis. Journal of Dentistry. 2011; 39:470–7.

27. Mazzoni A, Mannello F, Tay FR, et al. Zymographic analysis and characterization of MMP-2 and -9 forms in human sound dentin. Journal of Dental Research. 2007; 86:436–40.
28. Mazzoni A, Papa V, Nato F, et al. Immunohistochemical and biochemical assay of MMP-3 in human dentine. Journal of Dentistry. 2011; 39:231–7.
29. Mazzoni A, Pashley DH, Nishitani Y, et al. Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentine by etch-and-rinse adhesives. Biomaterials. 2006; 27:4470–6.
30. Mazzoni A, Pashley DH, Tay FR, et al. Immunohistochemical identification of MMP-2 and MMP-9 in human dentin: correlative FEI-SEM/TEM analysis. Journal of Biomedical Materials Research Part A. 2009; 88:697–703.
31. Nagase H, Woessner JF, Jr. Matrix metalloproteinases. The Journal of Biological Chemistry. 1999; 274:21491–4.
32. Nuttall RK, Sampieri CL, Pennington CJ, et al. 2004. Expression analysis of the entire MMP and TIMP gene families during mouse tissue development. FEBS Letters. 563:129–34.
33. Palosaari H, Pennington CJ, Larmas M. Expression profile of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs in mature human odontoblasts and pulp tissue. European Journal of Oral Sciences. 2003; 111:117–27.
34. Sa Y, Hao J, Samineni D, et al. Brain distribution and elimination of recombinant human TIMP-1 after cerebral ischemia and reperfusion in rats. Neurological Research. 2011; 33:433–8.
35. Santos J, Carrilho M, Tervahartiala T, et al. Determination of matrix metalloproteinases in human radicular dentin. Journal of Endodontics. 2009; 35:686–9.
36. Sartor L, Pezzato E, Dell'Aica I, Caniato R, et al. Inhibition of matrix-proteases by polyphenols: chemical insights for anti-inflammatory and anti-invasion drug design. Biochemical Pharmacology. 2002; 64:229–37.
37. Sluijter JP, de Kleijn DP, Pasterkamp G. Vascular remodeling and protease inhibition--bench to bedside. Cardiovascular Research. 2006; 69:595–603.
38. Sulkala M, Larmas M, Sorsa T, et al. The localization of matrix metalloproteinase-20 (MMP-20, enamelysin) in mature human teeth. Journal of Dental Research. 2002; 81:603–7.
39. Tay FR, Pashley DH, Loushine RJ, et al. Self-etching adhesives increase collagenolytic activity in radicular dentin. Journal of Endodontics. 2006; 32:862–8.
40. Tjaderhane L, Larjava H, Sorsa T, et al. The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. Journal of Dental Research, 1998; 77:1622–9.
41. Tjaderhane L, Palosaari H, Wahlgren J, et al. Human odontoblast culture method: the expression of collagen and matrix metalloproteinases (MMPs). Advances in Dental Research. 2001; 15:55–8.
42. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function and biochemistry. Circulation Research. 2003; 92:827–39.
43. Woessner JF, Jr. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. FASEB J. 1991; 5:2145–54.
44. Yu WH, Yu S, Meng Q, et al, 2000. TIMP-3 binds to sulfated glycosaminoglycans of the extracellular matrix. The Journal of Biological Chemistry. 275:31226–32.