

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

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Date of Receiving: 27/10/14

Date of Submission: 03/11/14

Date of Acceptance: 24/11/14

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 $400\mu\text{m}$.

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

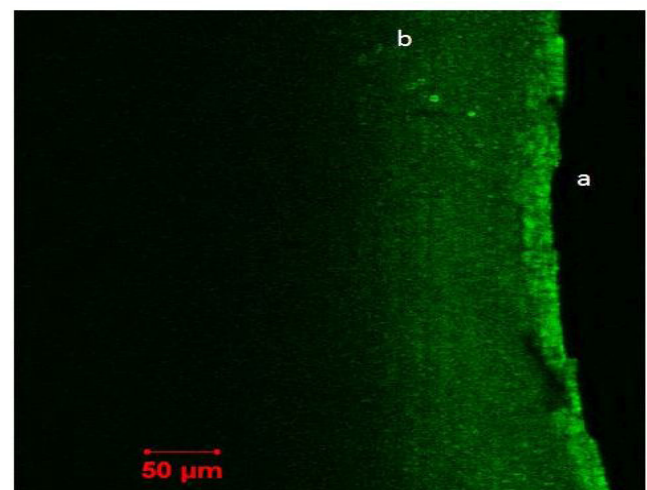


Figure 1. TIMP-1 in the normal radicular dentine.

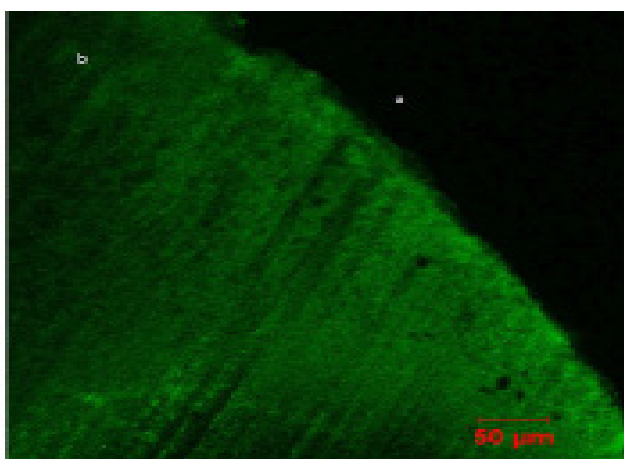


Figure 2. TIMP-2 in the normal radicular dentine.-

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

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