UTILISING *IN SITU* ZYMOGRAPHY AND FLUORESCENT IMAGING TO DETECT MATRIX METALLOPROTEINASES ACTIVITY WITHIN THE DENTINE

DR ALI A ALHARBI, **ALKARJ MILITARY HOSPITAL, KSA**

Introduction

Proteolytic activity of matrix metalloproteinases (MMPs) has been linked to degradation of the dentine-resin bond [(1)]. These MMPs can be released from the dentine and activated by the acid in resin cements [(2)]. Several methods such as SDS-PAGE and Western Blotting, ELISA and zymography are used to identify this proteolytic activity. *In situ* zymography is a variation of zymography where the enzymatic activity can be directly visualised on the dentine sample utilising fluorescence microscopy.

In this study, proteins conjugated to quenched fluorescein were applied to the dentine samples after bonding the samples to a self-adhesive resin cement. The proteolysis of these proteins by the enzymes in the sample released the fluorescence, which can be visualised by using fluorescence microscopy.

Methodology

Dentine slices were obtained from human permanent molars then covered with RelyX^{TM} Unicem Aplicap^{TM} self-adhesive resin cement (3M Company, Minnesota, USA) from one side. Other dentine slices obtained from the same teeth were left unbonded to the resin cement, to be used as negative control specimens.

As well as fluorogenic gelatine substrate solution, a negative-control (non-fluorescent) gelatine solution was also prepared. The solutions were applied to the dentine specimens then the specimens were inculpated in a light-protected humidified chamber at 37°C for 24 hours to permit for the enzymatic activity to take place.

Results

The obtained images have shown gelatinolytic activity in all of the dentine specimens bonded to RelyX^{TM} cement.

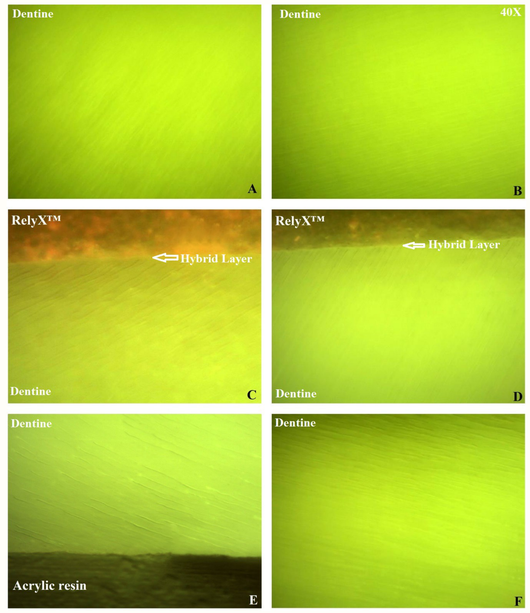

Figure 1. ***In situ*** **zymography negative control specimens. No fluorescence activity was observed under the fluorescence microscope (40X) in any of the images acquired from the negative control specimens (A-B Unbonded specimens treated with non-fluorescence gelatine. C-D RelyX^{TM} bonded specimens treated with non- fluorescence gelatine. E-F Unbonded specimens treated with fluorogenic gelatine).**

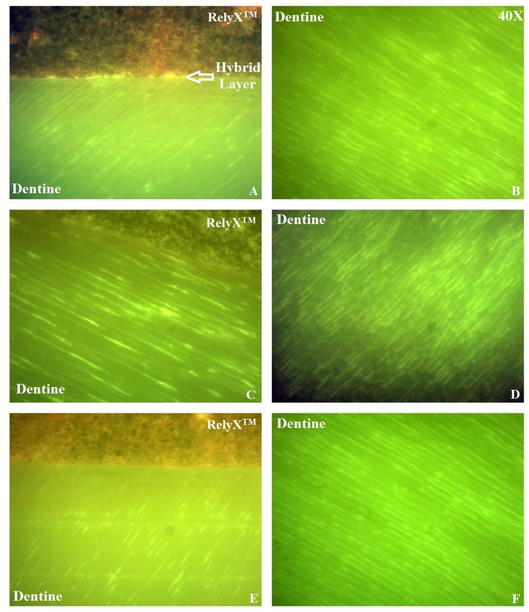

Figure 2. ***In situ*** **zymography test specimens. Varying degrees of fluorescence activity (indicated by bright green colouration on the images) were observed within the hybrid layer and/or along the dentinal tubules in all of the specimens bonded to RelyX^{TM} cement and treated with the fluorogenic gelatine substrate when viewed at 40X under the fluorescence microscope. Images A, C and E showing fluorescence activity along the hybrid layer between the RelyX^{TM} and dentine. Images A, B, C, D, E and F showing fluorescence activity within the dentinal tubules.**

Conclusion

This method is a very unique method that enables accurate localisation of matrix-degrading MMPs activity in dentine sections.

References

1.Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol IL, Geraldeli S, Tezvergil-Mutluay A, Carrilho MR, Carvalho RM, Tay FR, Pashley DH. Optimizing dentin bond durability: control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. Dental Materials. 2013 Jan 1;29(1):116-35.

2.Sano H, Yoshikawa T, Pereira PN, Kanemura N, Morigamui M, Tagami J, Pashley DH. Long-term durability of dentin bonds made with a self-etching primer, in vivo. Journal of dental research. 1999 Apr;78(4):906-11.