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Utilizing *in situ* zymography and fluorescent imaging to detect matrix metalloproteinases activity within the dentine

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Proteolytic activity of Matrix Metalloproteinases (MMPs) has been linked to degradation of the dentine-resin bond. These MMPs can be released from the dentine and activated by the acid in resin cements. 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 visualized on the dentine sample utilizing fluorescence microscopy. In this study, proteins conjugated to quenched fluorescein were applied to the dentine samples after bonding the samples to self-adhesive resin cement. The proteolysis of these proteins by the enzymes in the sample released the fluorescence, which can be visualized by using fluorescence microscopy. Dentine slices were obtained from human permanent molars then covered with RelyXTM Unicem AplicapTM self-adhesive resin cement (3M Company, Minnesota, USA) from one side. Other dentine slices obtained from the same teeth were left un-bonded 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 inculcated in a light-protected humidified chamber at 37 °C for 24 hours to permit for the enzymatic activity to take place. The specimens were viewed at 40X under a compound microscope with fluorescence attachment. Digital images of the specimens were obtained using an attached digital microscope camera. The obtained images have shown gelatinolytic activity in all of the dentine specimens bonded to RelyXTM cement. This method is a very unique method that enables accurate localization of matrix-degrading MMPs activity in dentine sections.

Biography

Ali A Alharbi has completed his PhD in Restorative Dentistry at the University of Dundee, School of Dentistry. He is the Director of Prosthodontics Division at Alkarj Armed Forces Hospital Dental Department.

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