An enhanced *in vitro* model to study the effectiveness of dentinal fluid on remineralization

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Abstract

Introduction: The Atraumatic Restorative Treatment (ART) is based on the indirect pulp capping technique. In these techniques, the infected dentine is removed and a glass ionomer cement (GIC) restoration is placed on remnant demineralized dentine to allow remineralization to occur. There is some evidence from *in vitro* and *in vivo* studies that fluoride and strontium diffuse into demineralized dentine (Kitasako, Nakajima et al. 2003); Smales 2005; Ngo 2006), and may contribute to remineralization. However, it is proposed that the mineral ions diffusing from dentinal tubular fluid also contribute. Shellis (1994) and Ozok (2004) recently demonstrated that infused surrogated dentinal fluid could decrease the depth of demineralization in dentine under an acidic challenge.

Objectives: This study aimed to investigate the role of Simulated Dentine Tubule Fluid (SDTF) in remineralization of the remnant demineralized dentine using an *in vitro* model of the ART technique.

Material and method: An apparatus was developed to achieve fluid flow into the pulp chambers of molar crowns, and thus into the dentine tubules at the same hydrostatic pressure as operates *in vivo*. A pilot study was carried out which demonstrated that the dye can diffuse into the dentine tubules when both artificial and natural caries are present, thus validating the method. A further pilot study demonstrated that SDTF could reduce the level of calcium lost in dentine under an acidic challenge, which agreed with Shellis and Ozok's findings. Moreover, in the presence of the SDTF, the remineralization of demineralized dentine increased with exposure to a remineralizing solution.

The main experiment of this study was to investigate whether SDTF can enhance remineralization of demineralized dentine using an *in vitro* ART simulation method. In this experiment, Class 1 cavities of similar dimension were cut in 18 extracted, intact third molar teeth. These teeth were painted with nail varnish leaving the dentine floors exposed for generation of artificial caries (6 teeth each for 7 days, 14 days and 21 days of

demineralization). Half of the demineralized cavity floor was protected with nail varnish providing a control and test side. The cavity was restored in Fuji IX which enabled a direct contact between demineralized dentine on the test half of the cavity floor and Fuji IX. A reservoir of SDTF was connected to supply SDTF under prescribed hydrostatic pressure to each pulp chamber for 21 days during which ion exchange was proceeding between GIC and demineralized dentine as demonstrated by Ngo (2005). Teeth were then detached from the system, sectioned and prepared for analysis by Electron Probe to determine profiles of Ca, P, F and Sr across the demineralized dentine under the GIC restoration. The results from this experiment, while showing an increase in calcium in the test over control side of the lesion, also showed an increase of calcium levels on both test and control side. This suggested a need to modify the model to provide evidence of the increase in calcium and other elements above the baseline profiles originally present in the demineralized dentine.

The modified model was developed which sectioned the teeth into halves. Each half of the tooth was treated as an individual sample in separated experiments where one was supplied with SDTF and the other with De-ionized Distilled Water. Based on the assumption that the mineral contents in two halves of the same tooth initially were similar, any increase in mineral contents in either half of the tooth could be considered as an indication of remineralization.

Other experiments were carried out to investigate the interaction between the SDTF to calcium rich materials which were applied to the demineralized dentine, and to investigate the effect of longer exposure time on remineralization. In the first experiment, Ketac Molar was used as an alternative restorative material which is calcium based restorative material. In the second experiment, CPP-ACP was applied to the demineralized dentine before placement of composite resin or glass ionomer cement restorations. In the third experiment, the exposure time of demineralized dentine under Fuji IX restoration to SDTF was increased to six weeks to look at the effect of longer periods of exposure to SDTF on overall remineralization.

Results: Both the initial and enhanced models showed the effectiveness of SDTF on remineralization *in vitro* under GIC restorations. The results provided evidence of an initial

positive equilibrium gradient between SDTF and demineralized dentine. The placement of a GIC restoration provided an added gradient in terms of calcium concentration between SDTF and the adjacent GIC restoration. If the calcium level in the restoration was higher than that in the SDTF, as occurred with Ketac Molar, the diffusion of calcium into demineralized dentine was diminished. This also occurred in the presence of CPP-ACP. In the presence of CPP-ACP, the calcium/phosphorus ratios in the demineralized dentine (adjacent to the restoration) was reduced and nearer to the ratio in calcium deficient apatite. However, the longer exposure time of the demineralized dentine to GIC restoration in the presence of the SDTF increased remineralization.

Conclusion: Dentinal tubule fluid contributes significantly to remineralization of the partially demineralized dentine in this model of the ART technique. However, the level of remineralization is very sensitive to factors affecting the concentration gradients between critical structural elements with the SDTF and the restoration.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and to the best of my knowledge and belief, contains no material previously published or written by another person except where due references have been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library being available for loan and photocopying.

Signed

Vu Thanh My Anh Date :

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