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Short Communication

Comparison of Cervical and Mid Coronal Dentine Using a Desensitizing Bioactive Glass Toothpaste: A Pilot Study

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Abstract

Aim: The aim of the present studywas to compare the effect of a calcium sodium phosphosilicate (NovaMin®) desensitizing agent (a bioactive glass) on both cervical and mid-coronal dentine sections using scanning electron microscopy (SEM).

Methods: Sections of both cervical and mid coronal dentine were prepared and etched with 5% citric acid for 5 minutes; these sections were then brushed with 2mg of Sensodyne Repair and Protect (NovaMin®) for 2 minutes with a powered toothbrush (Oral B, Braun) and left in artificial saliva (AS) for an hour at 37° degrees. One group, a control, was brushed only with distilled water and another group was subjected to a one-minute acid challenge with 5% citric acid. Samples were then prepared for anaylsis with SEM.

Results: The dentinal tubules identified on the root surface were narrower and less numerous compared to a mid-coronal section. In the test group, there was evidence of bioglass occlusion in 100% of the tubules after treatment.After the acid challenge approximately 6% of tubules were not occluded at all and 42% were only partially occluded with less than 50% of the diameter of the tubule closed.

Conclusions: The bioactive glass toothpaste (NovaMin®) appeared to occlude all of the dentinal tubules by more than 50% of their diameter and partially resisted an acid challenge on the cervical dentine section. The use of a cervical dentine section appeared comparable to the images of mid coronal dentine and would be more relevant for the evaluation of potential desensitizing products in the treatment of Dentine hypersensitivity. The sectioning of cervical dentine however may be more challenging than the sectioning of mid coronal dentine.

INTRODUCTION

Dentine hypersensitivity (DH) occurs when the dentinal tubules are exposed to the oral environment through the loss of overlying tooth structure either through tooth wear processes and/or through erosive elements in the diet. DH is a common condition, a recent study reported that the prevalence of DH in young adults in Europe was 42% [1]. Traditionally DH has been defined as a brief, sharp pain arising from exposed dentine in response to thermal or osmotic stimului which cannot be explained as arising from any other dental defect or pathlogy (disease) [2]. The buccal cervical region of the tooth is a common site for DH, which may be related to over-zealous or incorrect brushing technique with an abasive toothpaste. According to Addy et al., (1987) subjects with meticulous oral hygiene with high levels of plaque removal may be related to an increase in DH [3]. The mechanism underlying DH has been explained by the hydrodynamic theory [4], for example when a stimulus is applied to dentine it causes an increase in the rate of fluid flow through the dentinal tubules, which distorts the A-beta and A-delta fibres [5]. One study observed eight times the number of exposed tubules with twice the average diameter in sensitive teeth in comparison to assymptomatic teeth; which provided some support for the hydrodynamic theory [6]. The aetiology of DH is principally the exposure of the dentinal tubules; either through the loss of enamel or cementum, typically due to multifactorial tooth surface loss (attrition, abrasion, erosion and abfraction), loss of gingival tissue overlying the tooth may also occur. The treatment of DH is through either the modification of the pulpal response or through the blocking of patent dentinal tubules, this

study will be investigating the later occurrence. The method of occluding tubules is based on the hydrodynamic theory, which is by preventing fluid movement inside the dentinal tubules, and thereby relieving symptoms of sensitivity [7]. Ideally, the occluding material must be able to withstand acidic challenges that typically occur in the oral environment.

Bioactive glasses were originally developed for bone regeneration [8] but subsequently they were investigated for their potential use as desensitizing toothpaste designed to block the dentinal tubules [9]. For example, desensitising toothpaste containing the original 45S5 bioglass, with calcium, sodium, phosphate and silica was demonstrated to block the dentinal tubules *in vitro* [10] as well as reduce DH in a clinical study [11]. The mechanism by which the bioglass particles react in the mouth would suggest that following an interaction with saliva a series of chemical reactions result in the formation of a hydroxycarbonate apatite-like layer, which subsequently occluded the dentinal tubules [12]. Although NovaMin® toothpaste formulations have been evaluated in clinical trials for treatment of DH; data from clinical trials are still currently lacking [13].

Previous studies that have investigated potential desensitizing products have utilized a mid coronal section of dentine (the so-called dentine disc model) [14] however clinically DH can occur on the buccal (facial) aspects of the tooth for example, when the root surface is exposed following the loss of gingival tissue (gingival recession) [15,16]. One of the reasons for the popularity of the mid coronal section methodology is that the discs are easy to section whereas the sectioning of the cervical area of the tooth is technically more demanding. One however should be aware that the differences between mid coronal and cervical dentine have an impact clinically, for example previous studies have reported that cementum is less mineralized (61%) than enamel (95%) and is approximately 0.05 to 0.6mm compared to enamel (around 1.5.5-2.5mm thick) [17,18], which would suggest that it is more susceptible to tooth surface loss and subsequent tubule exposure. Furthermore, the differences in tubular density between mid coronal and cervical dentine [19-21] may also be important particularly when considering the rate of fluid flow through dentine as well as an effect on the ability of desensitizing products to be successfully applied onto the cervical area of the tooth.

MATERIALS AND METHODS

The Ethics Committee of the Queen Mary University London (QMUL) to enable the investigators to conduct the *in vitro* study in accordance with the Human Tissue Act granted ethics approval for the removal of human teeth.

The preparation of selected extracted teeth (n=10) for the mid-coronal dentine sections followed a standard process used in the Department based on the methodology used by Mordan et al. [22], described as follows: The dentine disc preparation procedure was initiated by fixing the tooth on a sample holder using Kerrs' impression material compound. The fixed sample was placed perpendicular to the cutting machine's blade. The cutting machine was a Malvern Instruments Microslice II cutting machine with a diamond-coated blade, (Model: BFC5-002). The blade was adjusted to cut every dentine disc in exactly 1 mm thickness. The enamel part was discarded and the cutting continued below the dentine-enamel junction in order to produce a disc containing only dentine. The section was then fracturered into two halves (test and control) using orthodontic pliers. For the cutting of the cervical sections, the procedure was modified following the removal of the crown section where the roots were sectioned lengthways with an automatic precision cutting machine (Figure 1). The section was fractured into a test and controls half prior to polishing and acid etching. As this was an experimental technique not routinely used in the Department a number of the sectioned teeth were not suitable for the *in vitro* study (e.g., caries observed in section). Following removal of any remaining cutting debris (smear layer) 0.8mm was removed from the outer surface of the tooth by polishing using a polishing machine (Kent 4 Automatic Lapping & Polishing Unit, Kemet International Ltd. Maidstone UK.) and silicon carbide grinding papers (USA). The polishing papers used were a fine P 400 paper, and a course grinding P 4000 paper. During the polishing procedure water was applied to the disk. Every dentine section was polished for 40 seconds; 10 seconds each side for both the P 400 and P4000 polishing papers. Subsequently, the polished dentine sections were cleaned using a Kerry Ultrasonic bath and the polished discs were placed in a beaker filled with deionized water and remained in the water bath for 5 minutes. Following polishing the test and control halves (n=6) were then etched with 5% citric acid for 5 minutes and stored in artificial saliva (AS) until required. The sections were initially brushed with 2mg of Sensodyne Repair and Protect (NovaMin®) which covered the disc surface for 2 minutes with a powered toothbrush (Oral B, Braun) and left in artificial saliva (AS) for an hour at 37°degrees. A subsequent acid challenge was applied to the sections using citric acid to minic the day-to-day challenge of dietary acids [23].

Sample A, the control, was brushed on its root surface for two minutes with a powered toothbrush (Oral B Braun) using distilled water, the section was then left in artificial saliva (AS) for an hour at 37^0 degrees. **Sample B** was brushed on its cervical surface for two minutes with a powered toothbrush with 2mg NovaMin® toothpaste and left in AS for an hour at 37° degrees. **Sample C** was treated identically to sample B but was subsequently given an acid challenge of 5% citric acid for one minute and then rinsed with distilled water.

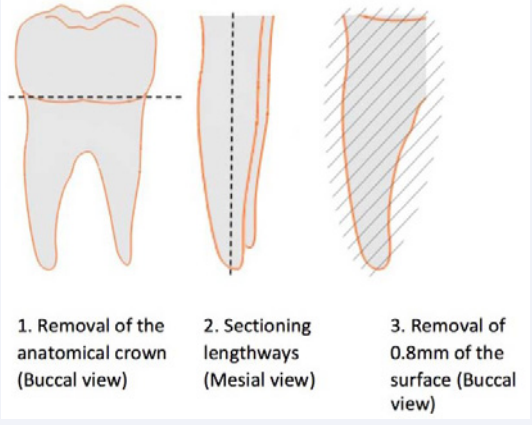

Figure 1 Sectioning of tooth samples.

The AS was prepared to pH 6.5 by dissolving 2.24g of KCl, 1.36g of KH_2PO_4, 0.76g NaCl, 0.44 g of $CaCl_2.2H_2O$, 2.2g of type II Mucin from porcine stomach (all weighed to within ± 0.001g) in 800ml of deionised water for a 1 litre batch, which was kept warm at 50°C. Each batch of AS was always used within a week of preparation (Table 1).

A gold coating was applied to the samples to reduce charging and dentine morphology was investigated using a scanning electron microscope (SEM).The Samples were imaged on an Inspect F (The FEI Company) under 5.00 KV and a 30 micron aperture, using a secondary electron detector. The regions of interest were determined in the following manner: 1) for the mid-coronal sections the area in the centre of the test half with its matching control half was investigated as described by Mordan et al. [22], 2) for the cervical section, a similar methodology was used with a control and test half. Images were taken at a working distance of approximately 7.5mm and at a magnification of x5,000 and x20,000.

RESULTS

The total number of the dentinal tubules and their sizes were approximated from the various images captured in the regions of interest by the SEM (Figure 2). The proportion of the dentinal tubules occluded by more or less than 50% of their diameter was recorded.

Group A (Control)

The dentinal tubules identified on the cervical surface in the region of interest were narrower and sparser compared to a typical mid-coronal section. In a sample of 44µm by 44µm at 5,000x magnification 30 dentinal tubules were identified in the cervical dentine (region of interest) compared to 98 in mid-coronal dentine. These were measured to be approximately 0.92µm in diameter compared to tubules of 2.5 to 1.2µm found mid-coronally (Figure 3).

Group B (Novamin application)

There was evidence of bioglass deposits in every dentinal tubulewith the majority fully occluded after treatment and 100% occluded by more than 50% of their diameter. The higher magnification image shows that the dentinal tubules have been occluded over their outer surface with crystal-like depositions (Figure 2).

Group C (Acid challenge)

After the acid challenge approximately 6% of the dentinal tubules were not occluded at all; 42% were partially occluded with less than 50% of the diameter of the tubule closed and 52% were occluded with more than 50% of the diameter of the dentinal tubule closed. The higher magnification image shows that the inside of the dentinal tubules still had deposits of material even though the surface of the dentinal tubule had loss of deposit following exposure (Figure 2).

Table 1: Quantities used to produce 1 liter of artificial saliva.

		g/l
Potassium chloride	30mM	2.236
Calcium chloride di-hydrate	3mM	0.441
Potassium di-hydrogen orthophophate	10mM	1.361
Sodium chloride	13mM	0.759
Mucin	0.22%	2.2

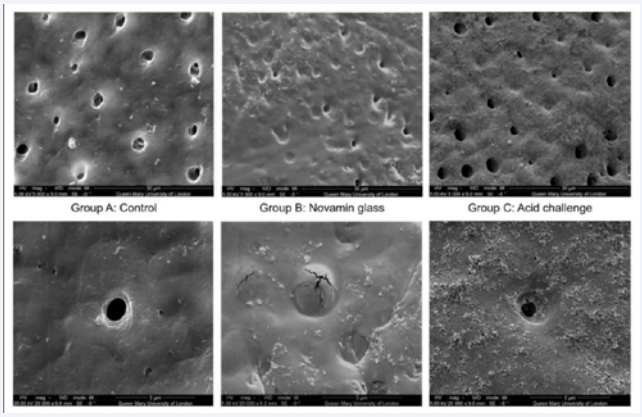

Figure 2 Images captured on SEM of control group and two best groups.

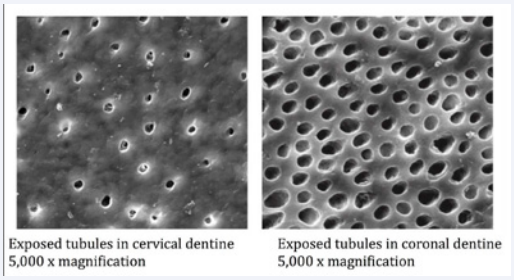

Figure 3 Comparison of cervical dentine and mid-coronal dentine.

DISCUSSION

The present *in vitro* investigation was a pliot study designed to determine whether using cervical dentine rather than mid-coronal sections would be a more appropriate technique for evaluating desensitizing products. It was limited in the number of samples that were used and no attempt to quantify the images using imaging software. Nevertheless, SEM imaging of the sections from the cervical area of the tooth (region of interest) demonstrated that the size and distribution of the dentinal tubules differed from those in mid-coronal sections, e.g., smaller in diameter and reduction in numbers. This observation to some extent supports the more detailed work by previous investigators comparing cervical and mid-coronal dentine of the tooth [6,20,21]. From these studies, it was evident that the tubule densities of both the inner and middle dentine of the root were significantly lower than that of the dentine in the crown that should be taken into account when investigating materials to be used in the mouth. *In vitro* studies attempt to mimic the oral conditions and therefore when investigating the ability of desensitizing products to occlude tubules, the rationale based on the Hydrodynamic theory is that DH occurs due to the presence of open dentinal tubules on the dentine surface and order to

mimic the oral environment the dental sections are acid etched prior to investigation following sectioning [9,10,14,22]. A further modification of this methodology includes the use of acid etching following the application of a desensitizing product on to the dentinal surface to mimic the effect of acid exposure in the mouth and to what degree the product is resistant to this challenge [23].

The present study has demonstrated that the application of a bioactive glass toothpaste (Novamin®) on both cervical and mid-coronal dentine resulted in the occlusion of the dentinal tubules. These results in terms of the surface depositon appeared to be similar to those reported in published studies using mid coronal dentinal sections [9,10]. In the present study NovaMin® was observed to occlude the majority of the dentinal tubules by more than 50% of their diameter and partially resisted an acid challenge in that there was sufficient remaining product in the dentinal tubules (in the region of interest) even though the surface deposit was removed. The advantage of using a test and control half from the same section of tooth is that one can observe the differences between the original acid etched control and its treated half, the control with its open tubules that are wide and free of deposit and the treated control which may have its surface covered with deposit and/or the majority of its dentinal tubules occluded with deposit [14,22]. The choice of desensitizing product that can both block the dentinal tubules and withstand an acid challenge is clearly of importance when developing desensitizing products for the treatment of DH.

Within the limitations of the present pilot study it was demonstrated that using cervical dentine specimens may be a more relevant methodology when evaluating desensitizing products. Although there were differences in the number of dentinal tubules and the width of these tubules in the respected regions of interest of both cervical and mid-coronal dentine, nevertheless these differences did not appear to impact on the observed surface deposit and the ability of a known desensiting product to block the dentinal tubules. Further research using a larger sample size and image analysis to quantify the results [24] however is required before advocating this methodology for the investigation of desensitizing products for the treatment of DH. The advantage of evaluating products however, in this model is that applying a desensitizing product using cervical dentine sections may be more relevant to the clinical situation where products are applied to the cervical region of the tooth rather than the mid-coronal region.

CONCLUSIONS

The bioactive glass toothpaste (NovaMin®) appeared to occlude all of the dentinal tubules by more than 50% of their diameter and partially resisted an acid challenge on the cervical dentine section. The use of a cervical dentine section appeared comparable to the images of mid coronal dentine and would be more relevant for the evaluation of potential desensitizing products in the treatment of DH. The sectioning of cervical dentine however may be more challenging than the sectioning of mid coronal dentine and further more detailed research is required before advocating this methodology for testing desensitizing products.

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