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Dentine desensitization induced by prophylactic and air-polishing procedures: An *in vitro* dentine permeability and confocal microscopy study

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ABSTRACT

Objectives: The exposure of dentinal tubules causes fluid movement and dentinal hypersensitivity. This study aimed at evaluating the dentine permeability after prophylactic measures performed on exposed dentine after immersion in artificial saliva and citric acid challenge. Confocal microscopy was performed to evaluate the percentage of occluded tubules (OCT%) and the changes in dentine morphology.

Methods: Prophy-powders and pastes were tested in this study. An oxalic acid liner was used as a positive control. Dentine discs from human third molars were treated with each material and the dentine permeability was evaluated using a fluid filtration system working at 20 cm H_2O. Artificial saliva and citric acid were used for the determination of changes in dentine permeability. The percentage of tubule occlusion capability (OCT%) was evaluated using confocal microscopy.

Results: All the products used in this study were able to significantly reduce the dentine permeability of acid-etched specimens. The use of the bioactive glass and sodium bicarbonate showed the highest values in dentine permeability reduction. However, the air-polishing procedures performed with Sylc bioactive glass powder created a dentine surface resistant to citric acid attack.

Conclusion: Bioactive glass is suitable for treatment of dentinal hypersensitivity by creating a dentine surface resistant to citric acid attack.

1. Introduction

Non-surgical periodontal therapy performed with sonic and manual instruments aims to remove plaque and calculus from the root surfaces to improve gingival health.[1–3] Nevertheless, the cementum in the cervical region and along the root is very thin, ranging from 20 to 50 μm even when intact and histologically normal, which can be easily removed during non-surgical periodontal therapy increasing the risk for root dentine hypersensitivity (RDH).[1,4]

The clinical symptoms of dentine hypersensitivity (DH) are principally caused by exposure of dentinal tubules as a result of enamel loss and/or gingival root surface exposure due to attrition, abrasion, erosion, abfraction or gingival recession.[5–7] It has been defined as a short sharp pain in response to thermal, evaporative, tactile, osmotic or chemical stimuli which may not be ascribed to any other form of dental defect or pathology.[5,6]

Based on Brännström's hydrodynamic theory,[8] dentine hypersensitivity is caused by movement of fluids within open dentine tubules. It is assumed that when a stimulus is applied

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on the exposed dentine surface it causes movement of tubular fluid, which in turn activates mechanoreceptor nerves, eliciting pain and discomfort.[9,10] Therefore, the occlusion of the tubules may reduce the fluid movement inside the dentinal tubules and the clinical symptoms of DH.[11] However, it is important to consider that if the occlusion of tubules is only superficial, daily tooth brushing, saliva or consumption of acidic beverages may easily open the dentinal tubules leading to short-term desensitizing effects.[12–14] In this regard, various desensitizing agents may be effective in occluding the dentinal tubules and reducing dentine permeability, unfortunately, little information is available on the effects of prophylactic measures on the DH.

Air-polishing devices such as Air-flow® (EMS, Nyon, Switzerland) or Prophyflex (KaVo, Germany) or Aquacut (Velopex, Horesham, UK) have become established for prophylactic treatments, enamel cleaning prior to pit and fissure sealing or orthodontic bracket bonding.[15–17] Moreover, these systems might also be used in the treatment of root surfaces during non-surgical periodontal therapy as a valid alternative to hand, sonic and ultrasonic scalers because these latter instruments are considered technically demanding and considered unpleasant by patients. Some authors have also affirmed that repeated use of these instruments may lead to hypersensitivity, weakening of the respective roots or even root fracture.[18,19]

The purpose of this study was to evaluate the changes in hydraulic conductance (i.e., dentinal permeability) after application of prophy-pastes or air-polishing powders during prophylactic procedures on exposed dentine immediately, after artificial saliva immersion and following final exposure to citric acid. A confocal scanning laser microscope (CLSM) was used to evaluate the percentage of occluded tubules (OCT%) and the changes in dentine morphology induced by experimental treatments. The null hypotheses tested in this study were that all the tested materials are able to reduce the dentine permeability at the same level and that citric acidic attack will induce no statistically significant change in dentine permeability reduction.

2. Materials and methods

2.1. *Specimen preparation for dentine permeability*

Human third molars (age 20–40) extracted for surgical reasons were collected and stored in deionized water (pH 7.4) at 4 °C prior to the experiments. All experiments were conducted within 1 month of extraction. Local protocols, reviewed and approved by the Ethics Committee of the Academic Health Science Centre at King's College London were followed, including informed consent for tissue use in research. Dentine crown segments were obtained by first removing the roots 1.5 mm beneath the cementum–enamel junction (CEJ) using a slow-speed water-cooled diamond saw (Labcut, Agar Scientific, Stansted, UK). The occlusal enamel of each crown segment was subsequently removed with a parallel cut to expose the deep dentine. Pulpal tissue was carefully removed from the exposed pulp chamber without damaging the pre-dentine surface by using thin tissue forceps. A pincer-type caliper was used for measuring the remaining dentine thickness (RDT) from the surface to the highest pulpal horns (0.7 and 0.9 mm). Each tooth section was attached to a Perspex™ (Perspex Distributions Ltd., London, UK) platform (2 cm × 2 cm × 0.5 cm) that was perforated by an 18 gauge stainless steel tube using cyanocrylate adhesive (ROCKET Heavy DVA, Corona, CA, USA). Each specimen was connected to a hydraulic fluid filtration system able to deliver a hydrostatic water pressure of 20 cm H_2O. A modified split-chamber device was used to allow the standardization of exposed dentine area for fluid filtration by using a rubber "O" ring with an internal diameter of 0.6 cm (area: 0.38 cm^2) (Fig. 1). A 25 ml capacity micro-capillary tube (Microcaps, Fisher Scientific, Atlanta, GA, USA) was positioned above a millimetric ruler and horizontally between the pressure reservoir and the crown segment. The linear displacement of an air bubble inside the micro-capillary tube, which indicated the volume displacement (hydraulic conductance), was detected using a high definition digital camera (Sony HDR-XR500V) placed vertically 5 cm above the micro-capillary and connected to a PC monitor *via* USB cable. The hydraulic conductance values were finally converted into the dentinal permeability (Lp): $Lp = Q/At$, where Lp is the dentine permeability ($\mu l\ cm^{-2}\ min^{-1}$), Q is the fluid flow (μl), A is the area of the dentine (cm^2), and *t* is the time (min).[11,20]

2.2. *Experimental design for dentine permeability*

A homogeneous smear layer was created on each dentine surface using a 500-grit abrasive paper for 30 s. Subsequently, the Lp was measured to evaluate the minimum permeability of each specimen. The smear layer was then removed by treating the dentine surface using 35% orthophosphoric acid for 30 s (PA). Subsequently, the dentine surface was copiously water-rinsed and the Lp was measured in order to obtain the highest permeability (Lp max = 100% was arbitrarily assigned). Using the Lp value as 100% flow permits evaluation of modifications in dentinal permeability following the test treatments. The specimens were then treated with the experimental products and the Lp of each specimen was measured after treatment. The percentage (Lp%) of flow after treatment was calculated by comparing the treatment Lp to the maximum Lp. Subsequently, the resistance of applied products to artificial saliva (AS) was tested by measuring the Lp after immersion of treated specimens in artificial saliva at 37 °C for 1 h under continuous stirring (120 rpm) (Table 1).

The composition of artificial saliva (in g/L) was $CaCl_2$ (0.103), $MgCl_2{\cdot}6H_2O$ (0.019), KH_2PO_4 (0.544), KCl (30) and HEPES (acid) buffer (4.77), the pH was 7.4. A 0.3% solution of citric acid titrated to a pH of 3.2 with NaOH buffer at 23.8 °C, was subsequently used for 5 min to test the ability of each treatment to resist to acidic challenge; evaluated by measuring the Lp of treated dentine after exposure to the citric acid solution and following water rinsing. Table 1 shows the number of teeth, specimens used per group and the experimental design steps. Evaluations of dentine permeability were performed by measurement of convective fluid flow through each crown segment under 20 cm H_2O of water pressure for 3 min in triplicate. The three convective fluid flow values were averaged to a single mean value. The average of the three runs for each sample and standard deviation of the dentine permeability (Lp) was calculated for each group. No