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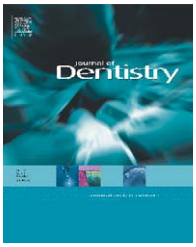

Dentine desensitization induced by prophylactic and air-polishing procedures: An *in vitro* dentine permeability and confocal microscopy study

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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^{TM} (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

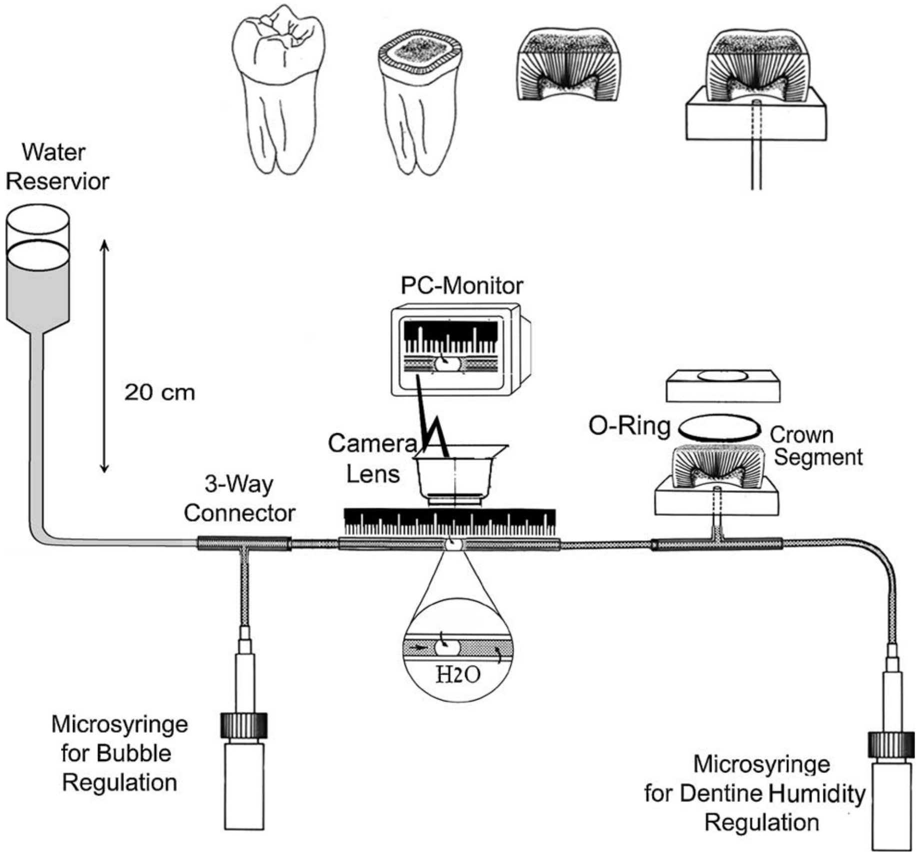

Fig. 1 – Schematic showing how crown segments were created, attached to Perspex™ and how fluid permeability was measured under 20 cm H_2O pressure.

positive hydrostatic pressure was applied to the dentine discs during the prophylactic procedures. All treatments were applied on a wet dentine surface. The dentine surface was rinsed using deionized water (30 s) after PA treatment and was kept moist to prevent dehydration.

The list of products used in this study, the composition and the application mode are shown in Table 2. The air-polishing system used with the different prophy-powders used in this study was Aquacut Quattro (VELOPEX International, London, UK) working at air pressure of 5 bar (500 MPa) and a distance of 10 mm.

Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA) software. The mean and standard deviation of each group were calculated from Lp% obtained from the treatments. As the normality of the data (Shapiro–Wilk W-test) and the equality of the group variances (Levene test) appeared to be valid and the statistical differences were identified among the groups by two-way ANOVA evaluating the effect of different prophylactic measures and the different challenges ($P < 0.01$). Fisher's least significant difference (LSD) test was used to isolate and compare the significant differences ($P < 0.05$) between the groups.

Table 1 – Experimental design for dentine permeability evaluation, number of specimens and list of the products of the principal groups.

Number of specimens per group (total number of teeth 40)		Treatments	Lp values
Principal group	Number of specimens		
Oxalate Liner	5	Production of smear layer by abrasive paper (30 s)	Lp minimum
Sylc bioactive glass® (air polishing)	5	Application of 35% PA (30 s)	Lp maximum (arbitrary value of 100%)
Sylc bioactive glass® (prophy cup)	5	Application of treatment (rubber cup or air-polishing)	Lp modifications
Cavitron® Prophy-Jet®	5		
AIR-flow® powder PERIO	5	Application of artificial saliva (1 h)	Lp increase
GC Tooth Mousse™	5		
Colgate® Sensitive Pro-Relief™	5	Application of citric acid (5 min)	Lp increase
NUPRO® NUSolutions™	5		

Table 2 – Products used in this study, composition and application mode.

Treatments (acronym)	Manufacturing	Components	Application mode
Oxalate Liner	Experimental solution	3 wt% monopotassium-monohydrogen oxalate in water [pH 2.7]	Applied for 30 s using an adhesive brush and then left undistributed on the dentine surface for 30 s
Sylc bioactive glass® (powder: air polishing)	SYLC, OSspray Ltd, London, UK	Bioactive glass (SiO_2, Na_2O, CaO, P_2O_5)	Applied to dentine using a slow-speed handpiece with a prophylaxis angle attachment angle and rotating rubber cup for 30 s
Sylc bioactive glass® (H_2O/powder: prophy cup)	SYLC, OSspray Ltd., London, UK	Bioactive glass (SiO_2, Na_2O, CaO P_2O_5) Deionized water	Air-polishing operated in a Prophyflex air-polishing device (Velopex) at a distance of 5 mm for 15s.
Cavitron® Prophy-Jet® (powder: air polishing)	DENTSPLY Corp., London, UK	Sodium bicarbonate ($NaHCO_3$)	Air-polishing operated in a Prophyflex air-polishing device (Velopex) at a distance of 5 mm for 15 s
AIR-flow® powder PERIO (powder: air polishing)	EMS Corp., Nyon, Swizerland	Amino-acid-glycine	Air-polishing operated in a Prophyflex air-polishing device (Velopex) at a distance of 5 mm for 15 s
GC Tooth Mousse™ (prophy-paste: prophy cup)	GC Corp., Tokyo, Japan	Pure water, glycerol, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), D-sorbitol, silicon dioxide, CMC-Na, propylene glycol, titanium dioxide, xylitol, phosphoric acid, guar gum, zinc oxide, sodium saccharin, ethyl p-hydroxybenzoate, magnesium oxide, butyl p-hydroxybenzoate and propyl p-hydroxybenzoate	Applied undiluted for 2 min on dentine surface using an adhesive brush. The excess was gently removed with deionized water
Colgate® Sensitive Pro-Relief™ (prophy-paste: prophy cup)	COLGATE PALMOLIVE, New York, NY, USA	Hydrated silica, calcium carbonate, glycerin, 8% arginine, water, bicarbonate, flavor, cellulose gum, sodium saccharin, FD&C blue no. 1 (CI 42090)	Applied to dentine using a slow-speed handpiece with a prophylaxis angle attachment angle and rotating rubber cup for 30 s
NUPRO® NUSolutions™ Prophy Paste (prophy-paste: prophy cup)	DENTSPLY Corp., London, UK	Hydrated silica, glycerin, water, bicarbonate, flavor, cellulose gum, sodium saccharin and bioactive glass Novamin®	Applied to dentine using a slow-speed handpiece with a prophylaxis angle attachment angle and rotating rubber cup for 30 s

2.3. *Specimen preparation for confocal microscopy*

Forty recently extracted molars were used for the confocal microscopy part of this study. Two 0.5 ± 0.1 mm thick dentine slices were obtained from deep-coronal dentine of each tooth using a slow-speed water-cooled diamond saw (Labcut, Agar Scientific, UK). The 80 slices were sectioned in two parts obtaining 160 hemi-slices that were randomly divided into 8 groups according to the number of the materials used in this study ($n = 20$/group) for examination with confocal microscopy. Each group was further divided in 4 subgroups ($n = 5$/group), according to the substrate treatment (i.e. Smear layer-covered dentine; 35%-PA; Product treatment; Artificial saliva/Citric acid challenge). Dentine slices were polished using a 500-grit abrasive paper to compose a flat dentine with standardized smear layer. The specimens were submitted to the same experimental treatment design as previously described in the dentine permeability evaluation section. The artificial saliva and the acid challenge steps were performed under continuous agitation at 120 rpm as previously described in the dentine permeability evaluation section and then copiously rinsed with deionized water (30 s).

The specimens were imaged using a confocal laser scanning microscope (Leica SP2 CLSM, Heidelberg, Germany) equipped with a 63×, 1.4 NA oil immersion lens using 514 nm argon/helium ion laser illumination. Reflected light was detected with a photomultiplier tube using reflection filters. A z-step of 1 μm was used to optically section the specimens to a depth of up to 30 μm below the surface. The z-axis scan of the dentine surface were converted into pseudo-color for better visualization, and compiled into both single and topographic projections using Leica SP2 CLSM image-processing software (Leica, Heidelberg, Germany) in order to evaluate the modifications induced by the experimental treatments. The configuration of the system was standardized and used at the same level for the entire investigation.

Each dentine slice was scanned and then 5 optical images were randomly captured to depths of up to 30 μm for a standardized area (59.52 μm × 59.52 μm). These images were intended to be representative of the surface features observed in each sample and to quantify the percentage of the occluded dentinal tubules resulting from different treatments (%OCT). This was measured and expressed as a percentage of the maximum number of open tubules obtained in each specimen etched using 37% H_3PO_4. The percentage of occlusion capability (%OCT) was calculated using the following equation:

$$\%\mathrm{OCT} = \frac{\begin{array}{c}\text{number of occluded tubules}\,(n/59.52\,\mathrm{mm}^2)\\ \text{in treated dentine surface} \times 100\end{array}}{\begin{array}{c}\text{maximum number of open tubules}\,(n/59.52\,\mathrm{mm}^2)\\ \text{in PA-etched dentine specimen}\end{array}}.$$

This represents the percentage of occlusion exhibited by the different treatments and products used in this study relative to its maximum number of open tubules obtained after H_3PO_4-

Table 3 – Intra-group comparisons of dentine permeability, occlusion capability evaluation and dentine permeability reduction after prophylactic treatment, immersion in saliva and citric acid challenge.

Lp%	Max Lp	Treatment	Artificial saliva	Citric acid
Oxalate Liner	100	8.5 ± 1.4^{A1} (δ −91.5) [0/0/100]	8.6 ± 1.4^{A1} $(\delta\ -91.4)^{a}$ [0/0/100]	8.9 ± 0.9^{A1} $(\delta\ -91.1)^{a}$ [1/5/94]
Sylc bioactive glass® (powder: air polishing)	100	12.6 ± 7.5^{AB1} $(\delta\ -87.4)^{a}$ [0/0/100]	12.9 ± 7.5^{AB1} $(\delta\ -87.1)^{a}$ [0/0/100]	18.9 ± 6.5^{B1} $(\delta\ -81.1)^{a}$ [2/7/91]
Sylc bioactive glass® (H_2O/powder: prophy cup)	100	18.2 ± 2.3^{B1} $(\delta\ -81.8)^{a}$ [3/7/90]	18.5 ± 2.3^{B1} $(\delta\ -81.5)^{a}$ [4/7/89]	29.1 ± 2.8^{C2} $(\delta\ -70.9)^{a}$ [11/32/57]
Cavitron® Prophy-Jet® (powder: air polishing)	100	17.3 ± 2.3^{B1} $(\delta\ -82.7)^{a}$ [0/0/100]	17.5 ± 1.9^{B1} $(\delta\ -82.5)^{a}$ [0/0/100]	27.8 ± 8.8^{C2} $(\delta\ -72.2)^{a}$ [5/10/85]
AIR-flow® powder PERIO (powder: air polishing)	100	31.9 ± 4.1^{C1} $(\delta\ -68.1)^{a}$ [3/8/89]	32.6 ± 4.2^{C1} $(\delta\ -67.4)^{a}$ [3/10/87]	42.2 ± 10.2^{D2} $(\delta\ -57.8)^{a}$ [15/37/48]
GC Tooth Mousse™ (prophy-paste: prophy cup)	100	51.4 ± 11.1^{D1} $(\delta\ -48.6)^{a}$ [11/32/57]	51.3 ± 11.2^{D1} $(\delta\ -48.7)^{a}$ [12/33/55]	58.7 ± 12.1^{E2} $(\delta\ -41.3)^{a}$ [40/40/20]
Colgate® Sensitive Pro-Relief™ (prophy-paste: prophy cup)	100	31.6 ± 5.5^{C1} $(\delta\ -68.4)^{a}$ [4/5/91]	31.8 ± 5.7^{C1} $(\delta\ -68.2)^{a}$ [5/10/85]	52.7 ± 4.4^{E2} $(\delta\ -47.3)^{a}$ [11/35/54]
NUPRO® NUSolutions™ Prophy Paste (prophy-paste: prophy cup)	100	32.5 ± 4.6^{C1} $(\delta\ -67.5)^{a}$ [13/12/75]	31.9 ± 5.1^{C1} $(\delta\ -68.1)^{a}$ [14/15/71]	53.7 ± 3.9^{E2} $(\delta\ -46.3)^{a}$ [19/34/47]

The values (expressed as (%)) are reported as means and standard deviations. Lp after 35% PA treatment was considered the maximum permeability (Lp = 100%). Same letter indicates no differences in columns with different product treatments maintained in the same challenge media. Same number indicates no differences in rows for different challenge media ($p > 0.05$).

[a] Delta values in parentheses indicate the percentage of dentine permeability reduction −Lp% between treatments and Max permeability (i.e. Max Lp; Treatment; Artificial saliva; Citric acid). Numbers in parentheses represent the percentage of tubules occlusion capability [completely open tubules (TOP) partially occluded tubules (POC) completely occluded tubules (TOC)].

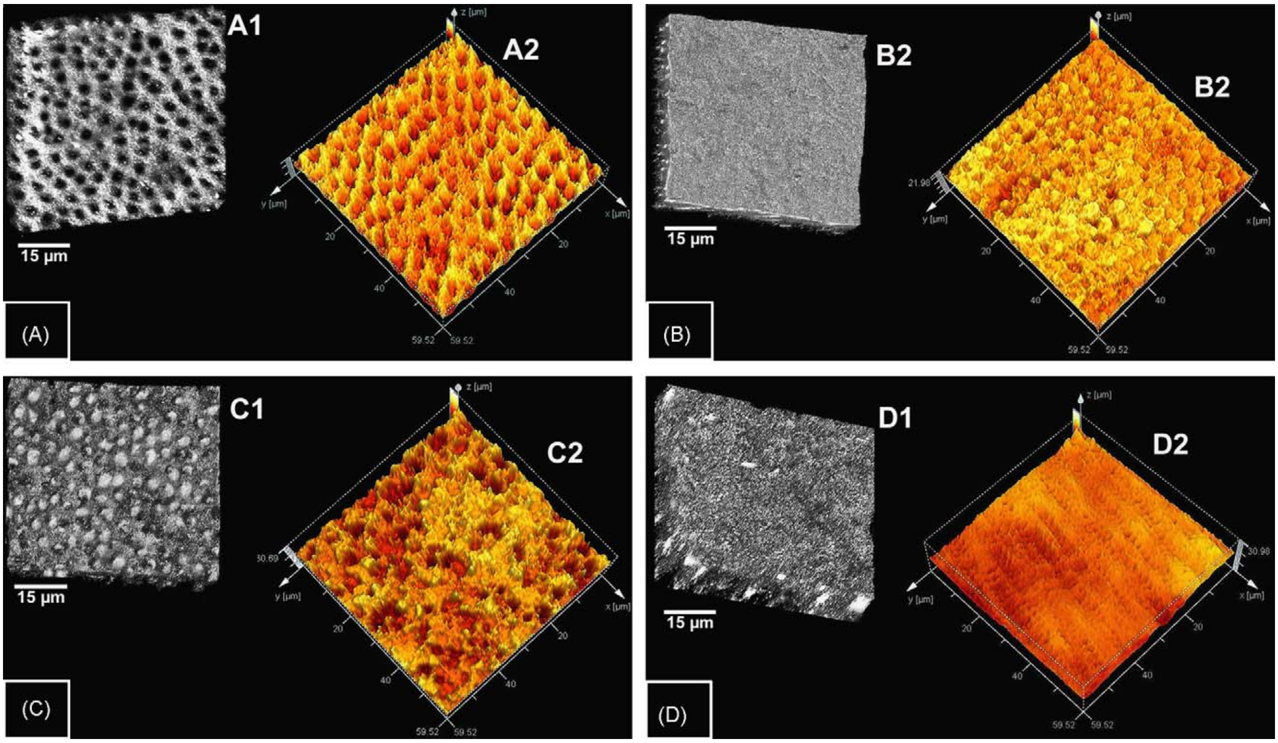

Fig. 2 – Confocal z-stack single projections and topographical reconstruction of dentine surfaces after application of the prophy-powders for air-polishing prophylactic procedures used in this study. Image A shows the effects of PA on the dentine surface. It is possible to observe a dentine surface characterized by completely open dentinal tubules (A1). In particular, A2 shows how PA induces many modifications in the intertubular and peritubular dentine. Image B shows the effects of bicarbonate powder used with the air-polishing system on the dentine surface. Images B1 and B2 show how this procedure creates a smear layer that covers the dentine surface and occludes the dentinal tubules. Image C shows the effects of EMS Perio powder on the dentine surface. Both in C1 and picture C2 is possible to observe a number of dentinal tubules completely or partially open showing how this procedure creates a smear layer that covers the dentine surface and occludes the dentinal tubules. Image D show the effects of Sylc-bioglass powder used with the air-polishing system on the dentine surface. Both D1 and D2 show how this procedure creates a multilayered smear layer that completely covers the dentine surface and occludes the dentinal tubules.

treated, with each dentine slice serving as its own control. The occlusion capability was finally expressed in Table 3 as the percentage of tubules completely open (TOP), tubules partially occluded (POC) and tubules occluded completely (TOC).

3. Results

3.1. *Dentine permeability after prophylactic procedures*

The dentine permeability results are expressed as means (%) and standard deviations in Table 3. All the products statistically reduced the maximum fluid flow of PA-etched dentine. Although artificial saliva induce no statistical differences in dentine permeability, several changes were observed after citric acid challenge (Table 3).

The treatment of dentine with 35%-PA increased the permeability to a maximum level which was arbitrarily considered equal to 100%.

The air-polishing procedures performed using Sylc bioactive glass and bicarbonate powders significantly reduced dentine permeability more than all the other treatments. However, the air-polishing procedures performed using Sylc bioactive glass (Lp 12.6%) showed no statistical differences when compared to the positive control (i.e. oxalate liner; Lp 8.5%). Conversely, oxalate liner gave dentine permeability values statistically lower than bicarbonate (Lp 17.3%).

The exposure of oxalate-treated dentine to citric acid did not significantly increase the dentine permeability (Lp 8.9%) when compared to the permeability of the oxalate-treated dentine. Likewise, the dentine permeability values of the specimens air-polished with Sylc bioactive glass (Lp 18.9%) did not statistically increase after acid challenge. On the contrary, sodium bicarbonate showed a statistical increase of the Lp up to 27.8% after citric acid challenge. The dentine air-polished with EMS Perio powder showed the highest permeability (Lp 31.9%) compared to the permeability obtained with the other air-polishing powders; citric acid challenge statistically increased the permeability up to 42.2%.

The dentine permeability of the specimens treated with the prophy-pastes showed many differences compared to those treated using the air polishing/prophy powders.

Sylc bioactive glass applied using a dental polishing cup with a rotary dental hand piece resulted in the lowest dentine

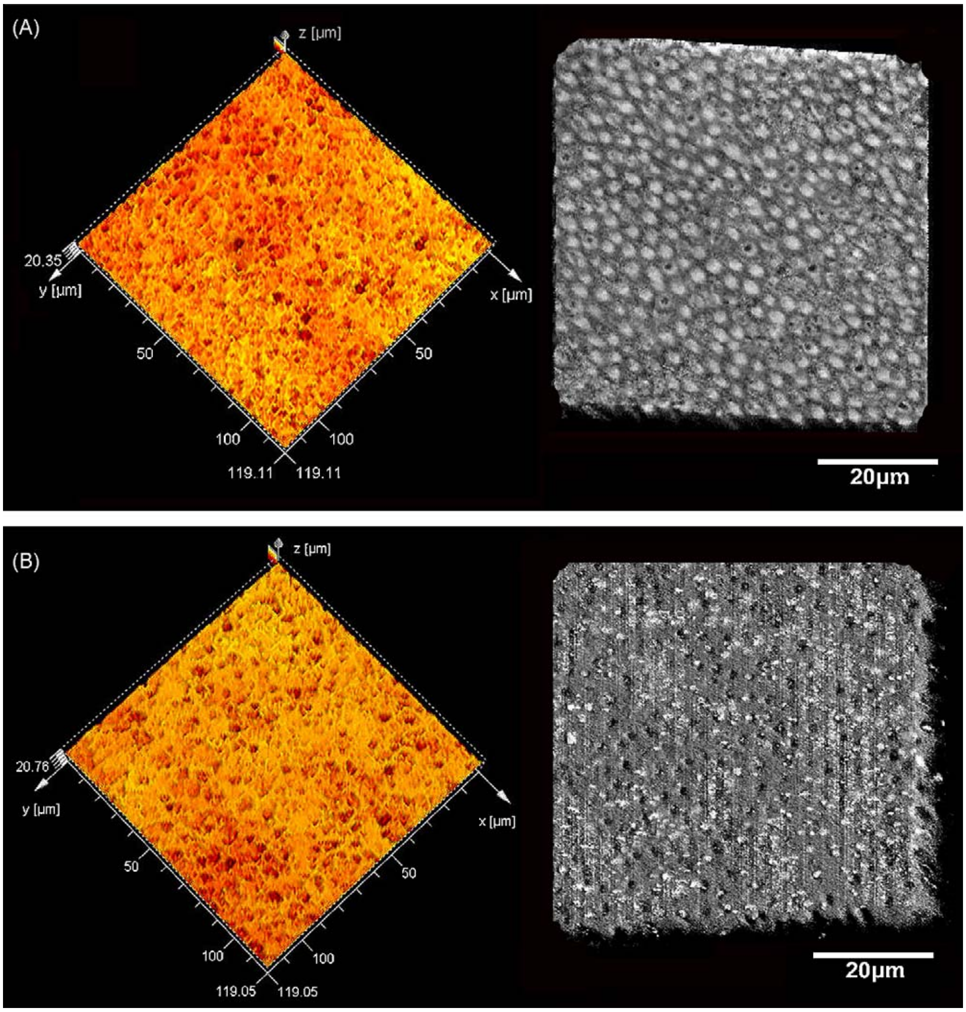

Fig. 3 – Confocal z-stack single projections and topographical reconstruction of dentine surfaces after application of the two prophy-paste for prophylactic procedures used in this study. Colgate Sensitive Pro-Relief: (A) the dentine surface appears covered by debris that occlude the dentinal tubules. Nupro Solution Prophy Paste: (B) the dentine surface appears superficially covered by residual debris that partially or completely occlude the dentinal tubules. It is possible to observe that some dentinal tubules are completely open.

permeability (Lp 18.2%) when compared to the other experimental prophy-pastes used in this study; citric acid challenge increased the dentine permeability up to 29.1%.

GC Tooth Mousse showed the highest dentine permeability values after application (Lp 51.4), artificial saliva immersion (Lp 51.3%) and after citric acid challenge (Lp 58.7%). When Colgate Sensitive Pro-Relief was applied on exposed dentine, the Lp was 31.6% and 52.7% after citric acid challenge. NUPRO Novamin-containing prophy-paste applied on the dentine surface had a dentine permeability of 32.5% that statistically increased up to 53.7% after citric acid challenge.

3.2. *Confocal microscopy and percentage of occlusion capability*

The tubules occlusion capability (%OCT), expressed as percentage of completely open tubules (TOP), partially occluded tubules (POC) and completely occluded tubules (TOC), are shown in Table 3. The oxalate-treated dentine, air-polished with Sylc bioactive glass or sodium bicarbonate showed that 100% of the dentinal tubules were totally occluded (Fig. 2A, B and D). The immersion in artificial saliva did not induce radical morphological changes. The citric acid challenge in the oxalate-treated specimens showed that 94% of the dentinal tubules were completely occluded (TOC) (Fig. 4A). The dentine air-polished with Sylc bioactive glass and subsequently immersed in AS and subsequently in citric acid showed that 91% of the tubules were still completely occluded tubules (TOC), while sodium bicarbonate-treated dentine showed 85% of completely occluded tubules. However, most of the smear layer was removed and the dentinal tubules were partially or completely exposed (Fig. 4B–D). The air-polished specimens using EMS Perio powder showed a percentage of the totally occluded tubules (TOC) of 89% after treatment, which decreased to 48% after citric acid challenge. The confocal imaging showed exposed dentinal tubules both after air-polishing treatment (Fig. 2C) and, in particular, subsequent to citric acid challenge (Fig. 4E).

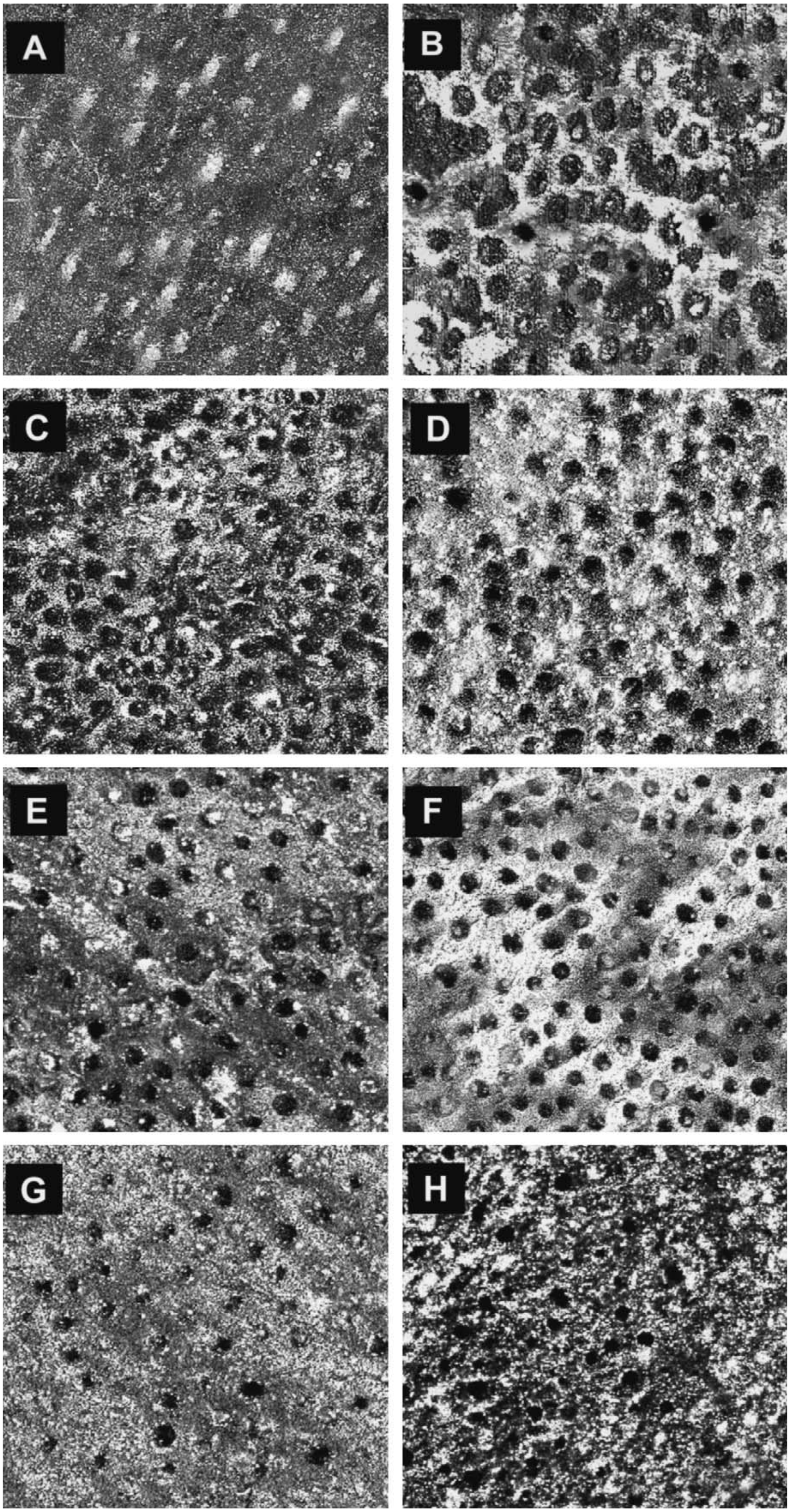

Fig. 4 – Confocal z-stack projections of dentine surfaces after application of tested materials and subsequently submitted to artificial saliva and citric acid challenge. Oxalate liner: (A) the dentinal tubules appear obliterated by crystal-like deposits. Sylc-bioglass powder: (B) the smear layer has been removed from the dentine surface but many tubules are still occluded by Bioglass/smear plugs. Bicarbonate powder: (C) the smear layer has been completely removed from the dentine surface and

Regarding the specimens treated with the prophy-pastes, the confocal microscopy showed many differences compared to those treated using the air polishing/prophy powders.

Sylc bioactive glass applied using a dental polishing cup showed that 90% of the tubules were completely occluded; the citric acid challenge left 57% of the dentinal tubules still occluded.

GC Tooth Mousse induced the complete occlusion of the tubules up to 20% and 40% of them were partially occluded (Fig. 4F). When Colgate Sensitive Pro-Relief was applied on exposed dentine 91% of the tubules were completely occluded. This percentage fell to 54% after citric acid challenge (Fig. 4G). The specimens treated with NUPRO Prophy Paste (Fig. 3H) showed that 75% of the tubules were still completely occluded and 47% after citric acid challenge.

4. Discussion

The aim of this study was the evaluation of the dentine permeability after prophylactic treatments performed with different prophy-pastes or prophy-powders using the Velopex air-polishing system on exposed dentine immediately, after artificial saliva immersion and after a citric acid challenge.

The *in vitro* evaluation of the efficacy of desensitizing agents, in agreement with the scientific literature,[7,10,14,20–22] may be performed using a fluid filtration system for hydraulic conductance measurement. Any substance that leads to a decrease in dentinal fluid conductance (i.e. dentinal permeability) by reducing the diameter or occluding the tubules and diminishing their number is able to reduce the symptoms of dentinal hypersensitivity.[7,10,23,24] This method was first developed and used by Pashley and co-workers[25] as a useful model to provide a valid evaluation of the ability of potential desensitizing agents to reduce fluid flow as a consequence of tubule occlusion.

Citric acid is a common component of fruit juices and soft drinks[26] and was used for the acid challenge in this study. Artificial saliva was used for *in vitro* studies to simulate a typical *in vivo* scenario for the evaluation of erosion and remineralization mechanisms[27] or for the evaluation of the risks for dentine hypersensitivity.[14,22]

Significant differences were observed in the results of dentine permeability of the specimens treated with the different types of product employed in this study. The oxalate liner used in this study was considered as the positive control for the reduction of dentine permeability. These results are in accordance with several *in vivo*[23] and *in vitro*[14,21,22,28,29] studies which indicated that desensitizers based on oxalic and/or potassium oxalate remain the gold standard treatment to occlude dentinal tubules by creating acid-resistant calcium oxalate crystals on the dentine surface and inside dentinal tubules (Fig. 4A). However, oxalic acid may have limitations as a clinical solution due to its potential toxicity. For instance, clinicians should avoid using oxalates in maxillary and mandibular trays for treating extensive dentine sensitivity because the oxalates are responsible for oral and gastric irritation.[30]

Air-polishing procedures have been employed for prophylactic measures for approximately 25 years. Over the past 15 years, these procedures have become more widely used for professional tooth cleaning as an alternative to pastes and rubber cup instruments.[31] The air-polishing system consists of air and powder applied to the dental surface using pressurized air at 4–8 bar (400–800 MPa) with or without the presence of water (1–5 bar; 100–500 MPa) leading to the removal of surface deposits, plaque and extrinsic discolorations. The prophy-powder commonly used with air-polishing systems is sodium bicarbonate ($NaHCO_3$) with a particle size less than 200 μm.

Recently, a softer prophy-powder based on the amino-acid, glycine, has been developed by EMS Corp. (Nyon, Switzerland) for both supra and sub-gingival applications.[32] Sylc is a bioactive prophy-powder and recently introduced to the market as an innovative prophy-powder for therapeutic air-polishing procedures. Sylc is a calcium sodium phosphosilicate powder, a highly biocompatible material which was originally developed as a bone conductive material.[33] The bioactive glass material reacts with body fluids (saliva) to deposit hydroxycarbonate apatite (HCA), a mineral that is chemically similar to natural tooth mineral.[33,34]

Sylc bioactive glass showed very high dentine permeability reduction both when it was used for air-polishing procedures (δ −87.4%) and when applied with a dental rubber cup as prophy-paste (δ −81.8%) (Table 3).

The sodium bicarbonate powder applied on exposed dentinal tubules reduced the dentine permeability up to −82.7% showing no statistically significant differences when compared to Sylc bioactive glass as prophy-powders. These two prophy-powders used for air-polishing procedures produced a smear plug deposited inside the dentinal tubule and a smear layer on the dentine surface (Fig. 2B and D) that occluded 100% of the exposed tubules that were observed in the PA-treated specimens (Fig. 2A).

However, when sodium bicarbonate was applied to the dentine specimens and subsequently submitted to artificial

many tubules are open or partially occluded. Sylc-bioglass paste: (D) in this case the smear layer has been removed from the dentine surface and a number of dentinal tubules are completely open. Bioglass/smear plugs can also be seen obliterating the various dentinal tubules. EMS Perio powder: (E) also in this case the smear layer was completely removed from the dentine surface and several dentinal tubules can be observed completely open. It is also possible to observe a few dentinal tubules partially obliterated. GC Tooth Mousse: (F) the dentine surface appears smooth with no presence of a smear layer. It is possible to observe that most of the dentinal tubules are completely open and very few dentinal tubules are partially obliterated by residuals of the products. Colgate Sensitive Pro-Relief: (G) the dentine surface appears covered by residual debris that partially or completely occlude the dentinal tubules. It is possible to observe that citric acid exposed a number of dentinal tubules. Nupro Solution Prophy Paste: (H) most of the dentinal tubules were exposed by the action of the citric acid. Few dentinal tubules are only partially occluded by debris.

saliva and citric acid challenge, the dentine permeability reduction was statistically lower (−Lp 72.2%) than that observed in bioactive glass-treated dentine (−Lp 81.1%). A possible explanation for these differences observed between Sylc bioactive glass and sodium bicarbonate prophy-powders may be attributed to the different morphological characteristics of the smear layer created during the air-polishing treatments. Indeed, the Confocal microscopy imaging showed that the smear layer created by sodium bicarbonate was thinner with the presence of porosities (Fig. 2B) while, Sylc bioactive glass created a compact and substantially thicker smear layer (Fig. 2D) that was more resistant to the acid attack. The concept of a ''morphologically acid-resistant smear layer'' has been shown as previously described;[9] it was demonstrated that non-surgical treatments of scaling and root-planing performed using hand curettes were able to create a more compact and multilayered acid-resistant smear layer than the one created using ultrasonic device which was less compact with greater porosity. Further evidence can be observed in the results of the Sylc bioactive glass when used as prophy-paste applied on PA-dentine which showed that the smear layer was not thick and compact enough to resist to the acid attack. It was nearly completely removed from the dentine surface after citric acid attack, however, smear plugs were left inside several dentinal tubules (Fig. 4C).

The higher acid resistance of the smear layer created by Sylc bioactive glass may be also attributed to a possible remineralization of the smear layer occurring during artificial saliva immersion. Indeed, bioactive glasses facilitate hydroxyapatite deposition when exposed to fluids containing calcium and phosphate.[33,34]

The lowest dentine permeability reduction was observed in the group of specimens air-polished with EMS Perio (δ −68.1). These results are attributed to the presence of 11% of dentinal tubules completely (3%) or partially open (8%) (Table 3 and Fig. 2C). Recent studies have shown that powders based on glycine, due to their low abrasiveness, can be safely applied subgingivaly during maintaining periodontal treatment to remove both supra- and subgingival plaque thereby reducing the harm to root surfaces.[35,36,37] Glycine has amphoteric characteristics and a pH ranging from acidic to alkaline. Although glycine possesses the washing action of anionic surfactant and the bactericidal action of cationic surfactant, its acidic pH may negatively influence the capacity of reduction of dentine permeability in a wet environment.[39]

Indeed, it represents an alternative in both periodontal treatment as well as an air-polishing technology, but its efficacy as a dentine desensitizer remains relatively lower than the other powders which are able to create a reliable and more acid-resistant smear layer (Fig. 2C). Indeed, when a citric acid solution was used to assess the durability of dentine desensitizing treatments, the glycine-based prophy-powder (EMS Perio) also created the weakest acid-resistant surface showing only −57.8% of dentine permeability reduction and 37% of dentinal tubules were partially or completely open (15%).

Different sealing effects were observed in the specimens treated with the prophy-pastes used with the dental rubber cup. Although all the prophy-pastes used in this study were able to statistically reduce the dentine permeability of PA-treated dentine, these treatments were less effective in reducing dentine permeability than the bioactive glass materials delivered by the air abrasion system. The only exception, as previously described, was the Sylc bioactive glass when used as prophy-paste; the dentine permeability reduction of the PA-treated dentine (δ −81.8) was comparable to that obtained with the sodium bicarbonate. GC Mousse was least effective in reducing dentine permeability (δ −48.6) which showed 32% of the tubules partially and 11% completely open. The citric acid challenge increased the dentine permeability and the TOP% (20% completely open tubules; 40% partially occluded tubules) (Table 3 and Fig. 4F). These results are in accordance with a recent paper of Gandolfi et al.[38] who showed that dentine treated with GC Tooth Mousse was the most water permeable due to the presence of only little and fine debris on the dentine surface and inside the dentinal tubules. Moreover, they showed that dentinal tubules remained visibly open after citric acid challenge characterized by several alterations in peritubular morphology dentine.

The specimens treated with Colgate Sensitive Pro-Relief and NUPRO Novamin-containing prophy-paste showed a dentine permeability reduction of −68.4 and −64.5 respectively. A recent paper of Petrou et al.[39] showed that dentine specimens treated with either the 8% arginine–calcium carbonate toothpaste or the 8% arginine–calcium carbonate desensitizing prophylaxis paste (Colgate Sensitive Pro-Relief) were completely occluded with a dentine permeability reduction of approx 80% only after five applications. The results of this study showed that Colgate Sensitive Pro-Relief completely occluded 91% of the tubules (TOC) and the remnant dentinal tubules were partially (5%) or totally (4%) open. However, 54% of the dentinal tubules remained totally occluded after the citric acid challenge. Nupro NUSolutions™ is the only Prophy Paste containing NovaMin® with calcium and phosphate ions and it has been indicated for use to immediately relieve sensitivity. NovaMin® is the trade name for a calcium sodium phosphosilicate bioactive glass developed for use in oral health care. This study has demonstrated that citric acid challenge increased the dentine permeability of the dentine specimens treated with this product up to 53.7% due to the complete (19%) and partial (34%) exposure of the dentinal tubules. The percentage of dentinal tubules that resisted the citric acid challenge was 47%.

In conclusion, the null hypothesis that all the tested materials are able to reduce the dentine permeability to the same level must be partially rejected because sodium bicarbonate, Sylc bioactive glass used both with air-polishing systems and as prophy-pastes with rotary rubber instruments were the most effective to reduce the dentine permeability of PA-treated dentine compared to the other treatments which were less effective. However, the most effective products able to create a dentine surface resistant to citric acid attack is Sylc bioactive glass used with the air-polishing system. Therefore, the second null hypothesis that the partially demineralized dentine treated with the tested materials show no difference in dentine permeability after citric acidic attack must be rejected.

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