©Operative Dentistry, 2011, **36-2,** 222-230

Effects of Common Dental Materials Used in Preventive or Operative Dentistry on Dentin Permeability and Remineralization

S Sauro • I Thompson • TF Watson

CLINICAL RELEVANCE

The bioactive glass (Sylc) reacts with saliva depositing hydroxycarbonate apatite (HCA) within the demineralized collagen fibrils and occluding dentinal tubules. Therefore, it may be used as a suitable desensitizing bioactive material for the treatment of DH and as an air-cutting powder before bonding procedures to remineralize tooth structure and/or prevent further demineralization within the resin–dentin interface.

SUMMARY

The aim of this study was to evaluate the dentin remineralization induced by bioactive substances contained in common dental materials used in preventive and operative dentistry. Several materials were applied on human dentin segments. Dentin permeability was quantified using a fluid filtration system working at 20 cm H_2O. Micro-Raman, SEM-EDX, and microhardness calculation were used to evaluate changes in the mineralization of dentin. Dentin treated with the prophylactic materials showed different dentin permeability values, in particular subsequent to immersion in remineralizing solutions (RSS). The bioactive glass (Sylc) was the only substance able to reduce dentin permeability after immersion in remineralizing solution and to show hydroxyapatite precipitation as a sign of dentin remineralization. The reduction in dentin permeability obtained after the application of the other prophylactic mate-

*Dr. Salvatore Sauro, PhD, associate researcher, Biomaterials, Biometrics & Biophotonics, King's College London Dental Institute, Guy's, King's College and St Thomas' Hospitals, London, United Kingdom

Dr. Ian Thompson, PhD, senior lecturer, Biomaterials, Biometrics & Biophotonics, King's College London Dental Institute, Guy's Hospital, London SE1 9RT, United Kingdom

Professor Timothy F Watson, BSc, BDS, PhD, FDS, professor and chairman, Biomaterials, Biometrics & Biophotonics, King's College London Dental Institute, Guy's, King's College and St Thomas' Hospitals, London, United Kingdom

*Corresponding Author: Floor 17, Guy's Tower, London, SE1 9RT, England

DOI: 10.2341/10-225-L

rials used in this study was due to the presence of the remnant material in the dentinal tubules, with no remineralization effect after storage in remineralizing solution. In conclusion, the results indicated that bioactive glass prophy powder may induce immediate remineralization of dentin.

INTRODUCTION

Dentin hypersensitivity (DH) is caused principally by the movement of the intratubular fluid after the exposure of dentin due to enamel loss and/or gingival root surface exposure.[1-3] The consumption of acidic food and beverages may cause erosion of hard dental tissues and increase the risk for DH.[4,5] Indeed, an essential association exists between the frequency of ingestion of specific acidic foods and beverages with the exposure of dentinal tubules.[6-8] Furthermore, the tooth brushing subsequent to the assumption of dietary acids may enhance the exposure of the dentinal tubules and aggravate the DH.[7,9]

Desensitizing bioactive materials involved in the treatment of DH should be able to react with body fluids and/or saliva depositing hydroxycarbonate apatite (HCA) within the demineralized collagen fibrils and occluding dentinal tubules.[10,11] Mineralizing processes may also occur via the tubular fluid, which is very similar to the extracellular fluid and, in conjunction with the odontoblast processes, orchestrates mineralization processes.[12-14]

The aim of this article is to present a series of experiments performed to test the ability of bioactive substance contained in prophy pastes or air polishing/cutting powders in encouraging the dentin remineralization and occlusion of the dentinal tubules. The null hypothesis tested in this study was that all prophy pastes or powders containing bioactive principles are capable of occluding dentinal tubules and remineralizing the dentin tissue when immersed in a remineralizing saline solution (RSS) for 24 or 48 hours.

MATERIALS AND METHODS

Dentin Permeability Evaluation

Thirty-five human third molars recently extracted for surgical reasons were used in this study. Dentin crown segments were obtained by first removing the roots 1.0 mm beneath the cementum-enamel junction (CEJ) using a slow-speed water-cooled diamond saw (Labcut; Agar Scientific, Stansted, UK). The occlusal enamel was subsequently removed with a parallel cut to expose the deep dentin. Pulpal tissue was carefully removed from the exposed pulp chamber by using tissue forceps. The remaining dentin thickness (RDT) was 0.7 and 0.9 mm. The specimens were divided in seven groups (n=5) in accordance with the materials listed in Table 1. The specimens were positioned in a modified Perspex™ (Perspex Distributions Ltd, London, UK) split-chamber device with pairs of rubber "O" rings with an internal diameter of 5 cm to standardize the dentin surface for fluid filtration.[15] A 25-μL-capacity microcapillary tube (Microcaps; Fisher Scientific, Atlanta, GA, USA) was horizontally positioned between the pressure reservoir (20 cm H_2O) and the dentin surface. The hydraulic conductance obtained via a microcapillary tube was converted into the dentin permeability (Lp): Lp=Q/At, where Lp is the dentin permeability ($\mu L\ cm^{-2}\ min^{-1}$), Q is the fluid flow (μL), A is the area of the dentin (cm^2), and t is the time (minutes).[16]

A homogeneous smear layer was created using a 500-grit abrasive paper for 30 seconds to evaluate the minimum dentin permeability. The smear layer was then removed, treating the dentin surface using 35% orthophosphoric acid solution (PA) for 30 seconds, and the highest permeability (Lp max=100% arbitrarily assigned) was evaluated. Lp 100% permits evaluation of modifications in dentin permeability following the test treatments. The specimens of each group were then treated with the experimental products in order to calculate the dentin permeability and expressed as a percentage (Lp%) of the maximum Lp value (100%). Since the intent of this study was the evaluation of the remineralization of dentin and of the capacity to occlude the dentinal tubules by mineral formation, the specimens were treated by spreading the products using gentle strokes and a camel hair brush. Finally, the samples were gently rinsed to ensure removal of any excess product and stored in RSS for 24 and 48 hours to evaluate the effect of the remineralization on the dentin permeability. The RSS solution (in g/L) was $CaCl_2$ (0.103), $MgCl_2{\cdot}6H_20$ (0.019), KH_2PO_4 (0.544), KCl (30), and HEPES (acid) buffer (4.77), and the pH was 7.4. The RSS solution was replaced every 12 hours. In this study a 0.3% solution of citric acid (pH 3.2) was also used for 5 minutes to test the ability of each test sample to resist an acidic attack.[15]

Raman Microscopy Evaluation

Thirty-five dentin specimens (2×2 mm) with thickness of 1.5±0.1 mm were totally demineralized in 0.02 M citric acid (pH 3.5) for 72 hours under constant stirring (120 rpm/s) at 37°C. The specimens were then copiously rinsed with deionized water and

Table 1: *Dentin Permeability and Microhardness of the Tested Products*

Active Ingredients	Max Lp% (Etched Dentin)	Immediate Application			PBS, 24 h
		Dentin Permeability, %[a]	Dentin Permeability Reduction, %[b]	Microhardness of Dentin Surface[c]	Dentin Permeability, %[a]
3 wt% monopotassium-monohydrogen oxalate in water NaH C_2O_4 H_2O pH 2.7	100	9.5 ± 1.4 ^{A1}	δ −91.5	89.1 ± 1.5 ^{a1}	9.6 ± 1.5 ^{A1}
Bioactive glass 100% SiO_2, Na_2O, CaO P_2O_5 SYLC (Osspray Ltd, London, UK)	100	99.6 ± 5.2 ^{B1}	δ −0.4	90 ± 1.3 ^{a1}	21.3 ± 6.2 ^{A2}
Sodium bicarbonate NaHCO3 Cavitron® Prophy Powder (Dentsply Corp, London, UK)	100	98.3 ± 3.5 ^{B1}	δ −1.7	89.2 ± 1.1^{a1}	98.5 ± 3.7 ^{B1}
Amino-acid-glycine NH_2CH_2COOH EMS Perio (EMS Corp,) Nyon, Switzerland)	100	98.9 ± 4.1 ^{B1}	δ −1.1	90.1 ± 1.2 ^{a1}	91.6 ± 4.5 ^{B1}
CPP-ACP: casein and phosphopeptide-amorphous calcium phosphate § GC Tooth Mousse (GC Corp, Tokyo, Japan)	100	59.8 ± 9.5 ^{C1}	δ −40.2	88.9 ± 0.8 ^{a1}	56.8 ± 9.1 ^{C1}
8% calcium carbonate-arginine § Colgate Sensitive Pro-Relief (Colgate Palmolive, New York, NY, USA)	100	80.4 ± 6.5 ^{B1}	δ −19.6	89.9 ± 0.9 ^{a1}	79.8 ± 6.4 ^{D1}
5% calcium sodium phosphosilicate NovaMin® § NUPRO Solution Prophy Paste (Dentsply)	100	85.6 ± 4.5 ^{B1}	δ −14.4	88.9 ± 1.1 ^{a1}	76.5 ± 4.6 ^{D1}

[a] Reported as means. Lp after 35% PA treatment was considered the maximum permeability (Lp=100%).
[b] −Lp% between treatments and Max permeability.
[c] Numbers in brackets represent the microhardness of the dentine surface [KHN].
Same uppercase letter indicates no differences in columns with different product treatments maintained in the same media. Same number indicates no differences in rows for time of RSS immersion (p>0.05).
The products with the symbol § contain other ingredients mixed with the active principle. GC Tooth Mousse™: glycerol, D-sorbitol, silicon dioxide, CMC-Na, propylene glycol, titanium dioxide, xylitol, phosphoric acid, zinc oxide, sodium saccharin, ethyl p-hydroxybenzoate, magnesium oxide, butyl p-hydroxybenzoate, and propyl p-hydroxybenzoate; Sensitive Pro-Relief™: hydrated silica, glycerin, water, bicarbonate, flavor, cellulose gum, sodium saccharin; NUSolutions™: hydrated silica, glycerin, water, bicarbonate, flavor, cellulose gum, sodium saccharin.

treated with the experimental products as described above. Control specimens were stored in deionized water, while the dentin specimens treated with different products were immersed in the RSS solution for 24 and 48 hours.

Subsequent to the remineralization periods, the specimens were examined in wet condition using a computer-controlled confocal laser Raman apparatus equipped with a Leica DM/LM optical microscope with a 20× objective and CCD detector attached to a modular research spectrograph (Renishaw InVia; Renishaw plc, Gloucheshire, UK). A near-infrared diode laser spot size of ≤1 μm operating at 785 nm was used to induce the Raman scattering effect. The spectral coverage of this model ranges from 200 to 3000 cm^{-1}. The calibration of the wavelength and intensity was performed according to manufacturer's specification using a silicon standard and the calibration system integrated with the software (WiRE 3.2; Renishaw) . The entire dentin surfaces were examined with steps of 10.0 ìm on the X and Y axes using a computer-motorized stage and analyzed for the peak of hydroxyapatite at 961 cm^{-1} using the software Wire 3.2 (Renishaw).[20,21]

SEM-EDX Evaluation

Following the nondestructive procedure of Confocal Raman characterization, the specimens were im-

Table 1: *Dentin Permeability and Microhardness of the Tested Products (ext.)*

PBS, 24 h		PBS, 48 h			Citric Acid		
Dentin Permeability Reduction, %[b]	Microhardness of Dentin Surface[c]	Dentin Permeability, %[a]	Dentin Permeability Reduction, %[b]	Microhardness of Dentin Surface[c]	Dentin Permeability, %[a]	Dentin Permeability Reduction, %[b]	Microhardness of Dentin Surface[c]
δ −91.4	89.5 ± 1.4 [a1]	9.6 ± 1.6 [A1]	δ −91.4	89.5 ± 1.4 [a1]	9.8 ± 1.1[A1]	δ −91.2	89.8 ± 2.1 [a1]
δ −78.7	60.1 ± 3.5 [b2]	20.9 ± 5.9 [A2]	δ −79.1	57 ± 3.4 [b2]	38.9 ± 5.5 [B2]	δ −61.1	85.3 ± 1.3 [a1]
δ −1.5	86.7 ± 1.0 [a1]	97.3 ± 3.5 [B1]	δ −2.7	86.1 ± 0.9 [a1]	106.8 ± 6.5 [C1]	δ +6.8	89.1 ± 1.2 [a]
δ −8.4	88.9 ± 1.1 [a1]	93.4 ± 5.1 [B1]	δ −6.6	88.6 ± 1.1 [a1]	105.2 ± 5.5 [C1]	δ +5.2	89.9 ± 0.9 [a1]
δ −43.2	80.2 ± 1.1 [a1]	54.3 ± 8.5 [C1]	δ −45.7	78.9 ± 1.1 [a1]	84.2 ± 9.1 [D2]	δ −15.8	89.5 ± 1.0 [a1]
δ −20.2	88.1 ± 1.0 [a1]	79.1 ± 6.3 [D1]	δ −20.9	87.7 ± 1.1 [a1]	89.7 ± 5.5 [D1]	δ −10.3	89.1 ± 0.8 [a1]
δ −23.5	87.1 ± 0.9 [a1]	74.9 ± 4.5 [D1]	δ −25.1	87.7 ± 1.0 [a1]	85.1 ± 5.1 [D1]	δ −4.9	89.3 ± 1.0 [a1]

mersed in deionized water for 1 hour and then dehydrated, mounted on aluminum stubs, and sputter-coated with carbon. The morphology and microstructure of the specimens were analyzed using a Hitachi S3500 scanning electron microscope (Hitachi High Technologies, Maidenhead, UK) fitted with an Oxford Instruments Inca energy dispersive X-ray microanalysis system (EDX) (Oxford Instruments, Abingdon, UK) under conditions of 8 kV of accelerating voltage.

Microhardness Evaluation

Another thirty-five dentin discs (2×2 mm) with thickness of 1.5±0.1 were partially demineralized by immersion in a citric acid solution (0.02 M; pH 3.5) for 5 minutes. Microhardness, in terms of the Knoop hardness number (KHN), was performed using a Knoop indenter in a microhardness tester (Leitz Microhardness Tester; Ernst Leitz Wetzlar GmbH, Wetzlar, Germany) with a load of 100 g with a dwell time of 20 seconds. Each dentin slice was considered as a sample unit. Five measurements were randomly obtained in wet condition from each dentin surface and elaborated using the formula of the Method for Knoop Indentation Hardness of Advanced Ceramics (KHN=constant×test force/indent diagonal squared). The dimensions of all indentations were measured immediately following indentation to avoid possible shrinkage caused by mechanical recovery of the tooth surfaces.

Statistical Analysis

Statistical analysis was performed using the SPSS 16.0 (SPSS Inc., Chicago, IL, USA) program. Shapiro-Wilk W-test and the Levene test were used for the validation and normality of the results. Statistical differences were identified by two-way analysis of variance evaluating the effect of different prophylactic measures and the different challenges differences ($p<0.01$). Fisher's least significant difference test was used to isolate and compare the significant differences ($p<0.05$) between the groups.

RESULTS

Dentin Permeability Evaluation

The permeability of the PA etched-dentin specimens was arbitrary considered equal to 100% and represented the own control of each specimen subsequently treated with one of the experimental treatments.

All the products used in this study differently influenced the dentin permeability both immediately after the application and after RSS immersion (Table 1). For instance, 3 wt% monopotassium-monohydrogen oxalate induced the highest dentin permeability reduction (−91.5%). Subsequent to immersion in RSS and in citric acid, no statistical change was observed.

The bioactive glass, sodium bicarbonate, and amino-acid glycine powders applied on acid-etched dentin induced no statistical reduction in dentin permeability immediately subsequent to the application. Conversely, when the bioactive glass (Sylc) was applied on the demineralized dentin and subsequently immersed in RSS for 24 hours, a statistical reduction in dentin permeability was observed; the reduction of the dentin permeability after 48 hours of RSS immersion was −79.1%. Sodium bicarbonate (Cavitron Prophy Powder) and amino-acid glycine (EMS Perio) prophy powders showed no dentin permeability reduction after RSS immersion, and the citric acid attack increased the dentin permeability more than their own control (Table 1).

Regarding the application of the prophy paste containing casein/phosphopeptide-amorphous calcium phosphate (GC Tooth Mousse), a permeability reduction up to −40.2% was observed after the application, while the prophy paste containing 8% calcium carbonate-arginine (Colgate Sensitive Pro-Relief) and the prophy paste containing 5% calcium sodium phosphosilicate (NovaMin®; NUPRO Solution) showed a permeability reduction of −19.6% and −14.4%, respectively. However, these materials showed no further permeability reduction after RSS immersion; the citric acid attack increased the permeability of the treated dentin (Table 1).

Raman Microscopy Evaluation

The Raman spectroscopy showed that the sound dentin (control) was characterized by peaks spanning from 400 to 1100 cm^{-1} with the most prominent peaks at 961 cm^{-1}, which is representative of the mineral phase of the dentin ($\upsilon 1\text{-}PO_4^{3-}$; hydroxycarbonate apatite [HCA]).[17,18] Organic grouping vibration modes (amide and CH) representing the dentin collagen were detected in the 1200–3000-cm^{-1} region (Figure 1A-a). Conversely, totally demineralized dentin specimens showed no peaks in the region from 400 to 1100 cm^{-1}, indicating the absence of any phosphate group (Figure 1A-b) and a strong autofluorescence signal representing the organic components. The demineralized specimens treated with the bioactive glass (Sylc) and subsequently immersed in RSS showed peaks in the region from 400 to 1100 cm^{-1} with a prominent peak at 961 cm^{-1} (Figure 1A-c). The peak of HCA was not observed after application of sodium bicarbonate (Cavitron Prophy Powder) and RSS immersion, but, on the contrary, a strong signal representing the demineralized dentin collagen was clearly visible (Figure 1A-d). No HCA formation was detected during the Raman scanning both when the totally demineralized dentin specimens were treated with the other products and following the immersion in RSS for 24 and 48 hours (Figure 2B–E).

SEM-EDX Evaluation

The remineralization process with occlusion of the dentinal tubules were further investigated by scanning electron microscopy using an element-sensitive detector (EDX) to qualitatively record the element composition (Ca and P/O) within the dentin surface treated in accordance with the experimental design.[19] The control Ca and P/O ratios were recorded from the smear layer–covered dentin (Figure 2A). High Ca and P/O ratios were observed in this group of specimens (Figure 2a). Similar Ca/P and O ratios (Figure 2b) were detected from the specimens treated with the bioactive glass (Sylc) and immersed in RSS for 24 and 48 hours (Figure 2B). The application of sodium bicarbonate (Cavitron Prophy Powder) or amino-acid glycine (EMS Perio) with subsequent immersion in RSS did not induce any increase in the concentration of the Ca and P/O ratios of the dentinal tubules (Figure 2C,D).

Similar results were observed in the specimens treated with the prophy pastes; no particular increase in Ca and P/O ratios were detected from the tubules of these dentin specimens (Figure 3); a constant presence of silicon (Si) was instead observed (Figure 3c,d).

Microhardness Evaluation

The results of this experiment showed that the higher the value obtained by Knoop indentation hardness (KHN), the lower the superficial hardness of the dentin surface (Table 1). For instance, sound dentin (control) showed the highest superficial microhardness value (49.1). Conversely, dentin specimens totally demineralized showed a superficial microhardness value of 89.9 due to the visco-

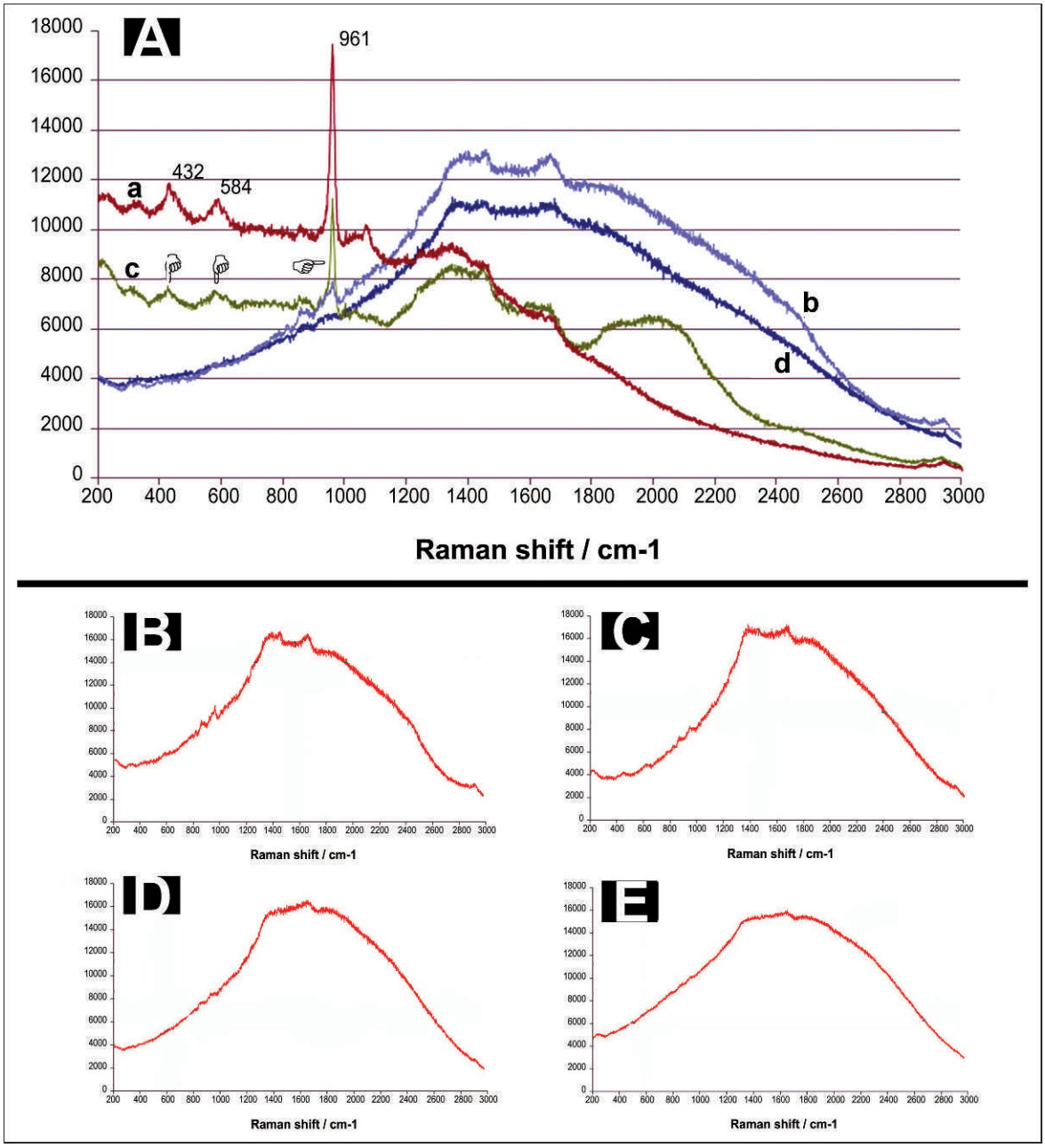

Figure 1. *Micro-Raman spectrums of dentin treated with the different remineralizing products and subsequently immersed in RSS for 24 and 48 hours. (A-a): Sound dentin is indicated by the presence of peaks at 432, at 584 cm^{-1}, and a peak with the highest intensity at 960 cm^{-1} representing the HCA component (pointers). (A-b): Totally demineralized dentin showing a Raman region spanning from 1200 to 3000 cm^{-1} representing the organic components of the dentin. (A-c): Raman spectra of the dentin treated with bioactive glass and immersed in RSS solution indicating the presence of peaks at 432, 584 cm^{-1}, and the highest peak at 960 cm^{-1} representing the formation and deposition of HCA. (A-d): Raman spectra of the dentin treated with CPP-ACP and immersed in RSS. It is possible to observe the presence of no peak for the HCA but only a high intensity region spanning from 1200 to 3000 cm^{-1} representing the organic components; the intensity of this region is slightly lower than that observed in the totally demineralized dentine (A-b). The same situation was observed in the specimens treated with sodium bicarbonate (B), arginine calcium-carbonate (C), Nupro containing 5% Novamin bioactive glass (D), and 100% amino-acid glycine (E).*

elastic characteristics of the demineralized dentin. Immediate application of the experimental products on the demineralized dentin showed no change in microhardness when compared to those obtained in the demineralized dentin specimens. Contrariwise, the immersion in RSS induced an increase of the superficial dentin microhardness only in those specimens treated with the bioactive glass (Sylc). No changes were observed in the specimens treated with the other products.

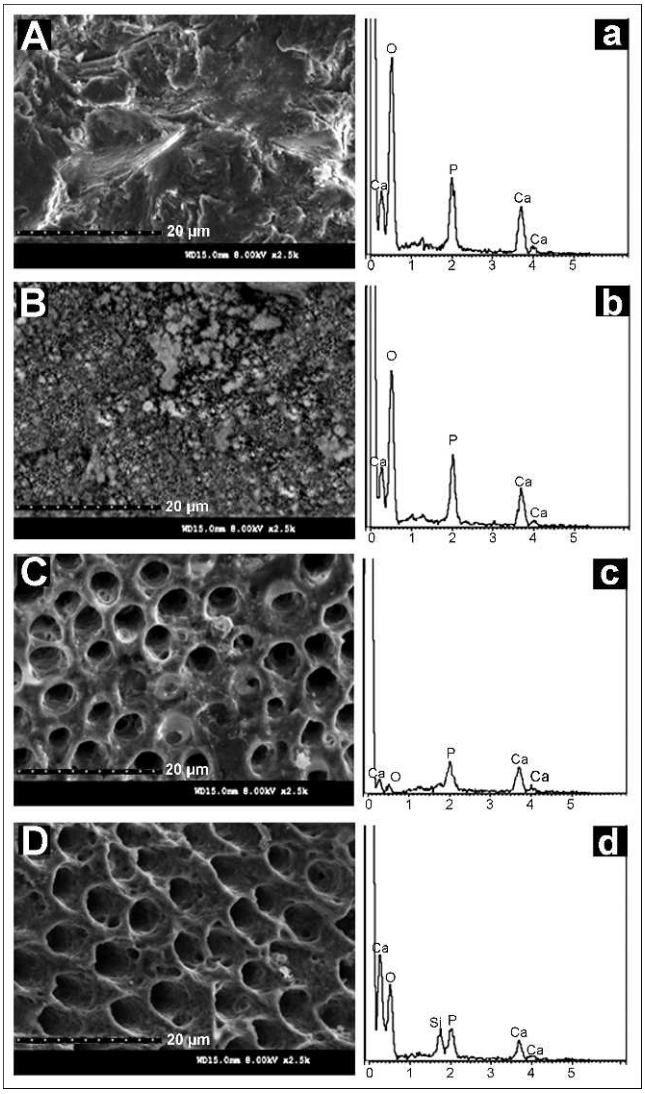

Figure 2. *SEM-EDX images of the smear layer–covered dentin (control), which shows no exposed dentinal tubule (A) and EDX spectra with high peaks of Ca and P/O characteristic of the mineralized dentin (a). The treatment of the demineralized dentin surface with bioactive glass (Sylc) and the immersion in RSS induced the precipitation of HCA (B). The EDX spectra of these specimens show presence of high Ca and P/O peaks comparable to those observed in the control specimens (b). The application of (Cavitron Prophy Powder) (C) or (EMS Perio) (D) induce no reliable HCA precipitation subsequent to RSS immersion (c, d).*

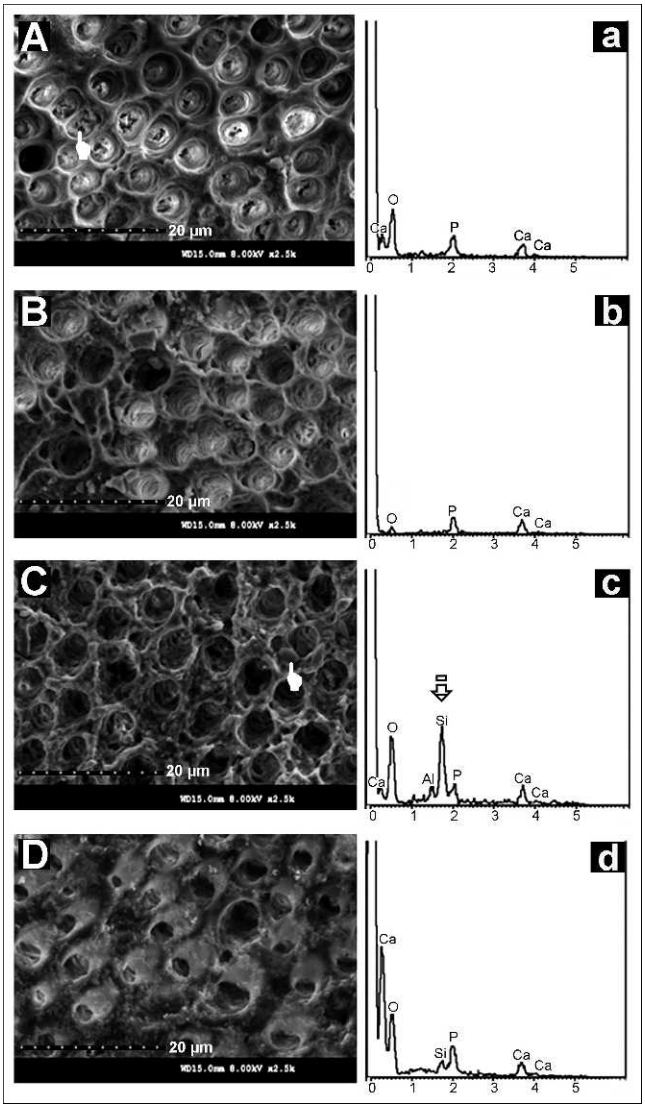

Figure 3. *(A): SEM-EDX micrographs of the dentin specimens treated with oxalic acid and immersed in RSS for 48 hours showing the presence of crystal-like deposits several microns deep inside the dentinal tubules (pointer). The EDX spectra shows low peaks of Ca and P/O on the dentin surface (a). The treatment of the demineralized dentin with Nupro NU Solutions™ and immersed in RSS induced no precipitation of HCA (B); the EDX spectra shows low peaks of Ca and P/O (a). (C): SEM-EDX micrograph of the dentin specimens treated with Colgate Sensitive Pro-Relief containing 8% arginine-calcium-carbonate and immersed in RSS for 48 hours showing the presence of many open dentinal tubules with very few debris on the dentin surface (pointer). Also in this case, the EDX spectra have low peaks of Ca and P/O (a) and a high presence of silicon (arrow) inside the dentinal tubules. The application of GC Tooth Mousse (D) induced no reliable HCA precipitation inside the tubules subsequent to RSS immersion but only a reduction of the lumen of the dentinal tubules (2–3 µm). Similarly, low peaks of Ca and P/O may be seen in this case (d) with the presence of silicon inside the dentinal tubules.*

DISCUSSION

The remineralization of dentin and the formation of crystalline apatite inside the dentinal tubules may be a reliable approach for the reduction of the dentin permeability in the clinical treatment of hypersensitivity.[11,12] Moreover, this concept may be considered for the reduction of the micropermeability within the hybrid layer of resin bonded dentin.[13] Many dental products used in preventive and operative dentistry have had manufacturer's claims that they can reduce the clinical symptoms of DH and induce dentin remineralization. Nevertheless, most of these materials are able to occlude the

dentinal tubule only via mechanical procedures that facilitate the formation of an artificial smear layer and smear plugs inside the tubules.[9,16] There is little information on the effective remineralizing potential of active components within the current clinical materials available.

The aim of this study was to perform a series of experiments to test the ability of bioactive substance contained in prophy pastes or air polishing/cutting powders in encouraging the remineralization and occlusion of the dentinal tubules. The experiments performed in this study have shown a significant dentin remineralization affect induced by the bioactive glass powder (Sylc) when compared to all the alternate test materials. Bioactive glasses encompass a wide range of clinical applications, some currently used as bone substitutes in periodontology, as load-bearing ceramic vertebral spacer prostheses, and as dentin desensitizing agents when used for air polishing procedures.[15,17] Bioactive glasses may also be used during restorative procedures in air-cutting/abrasion units as a substitute to alumina for an alternative nonmechanical cavity preparation. Its clinical use has many advantages, including reduced pain experienced by patients and selective removal of carious dentin and rounded internal cavity angle preparations that minimize stress concentration.[18]

The Raman microscopy evaluation showed that the demineralized dentin treated with Sylc bioactive glass and subsequently immersed in RSS for 24 and 48 hours was characterized by the reappearance of the peaks at 432, at 584 cm^{-1}, and a high intensity signal at 961 cm^{-1} (Figure 1Ac), indicating dentin remineralization.[19,20] The dentin remineralization observed in these specimens was confirmed by the microhardness results, which showed an increase in the superficial microhardness in demineralized Sylc bioglass-treated specimens immersed in RSS (Table 1). These results were not observed in any other group of specimens treated with the powders or prophy pastes used in this study. Indeed, there were no remineralization signals observed from any test material other than bioactive glass. The dentin spectra were comparable to those observed in totally demineralized dentin specimens, which showed a high intensity region spanning from 1200 to 3000 cm^{-1} (Figure 1Ab) representing the presence of demineralized dentin.[20]

The confirmation that the Sylc bioactive glass used in this study may be a suitable approach for the remineralization of dentin was also obtained by the evaluation of the dentin permeability. Indeed, the reduction of the dentin permeability was obtained only when exposed dentin was treated with Sylc bioactive glass powder and immersed in RSS for 48 and 48 hours (Table 1). Contrariwise, although the prophy pastes reduced the dentin permeability immediately after the application on demineralized dentin, no further reduction was observed after RSS immersion.

SEM-EDX investigation showed a spectrum with low peaks of Ca and P/O and a constant presence of silicon peaks inside the dentinal tubules (Figure 3). These results demonstrate that the reduction in dentin permeability observed in the specimens treated with the prophy pastes used in this study was induced by the occlusion of the tubules by the penetration of material plugs and not via HCA precipitation.[21] Conversely, SEM-EDX showed that the Sylc bioactive glass triggered the precipitation of HCA into the tubules and on the dentin surface (Figure 2B-b). The remineralization process induced by the Sylc bioactive glasses was due to a simultaneous biomimetic process, characterized by silicic acid $Si(OH)_4$ release, and a subsequent polycondensation reaction induced by precipitation of calcium and phosphates. Indeed, when calcium sodium phosphosilicate is immersed in a fluid analogous to saliva or body fluids, sodium ions (Na^+) immediately begin to exchange with hydrogen cations (H^+ or H_3O^+) within one minute.[22–24] This rapid exchange of ions allows calcium (Ca^{2+}) and phosphate (PO_4^{3-}) species to be released from the particle structure. A modest localized, transient increase in pH occurs and facilitates the precipitation of calcium and phosphate from the particles and from saliva to form an amorphous calcium phosphate layer ($CaO\text{-}P_2O_5$) on tooth surfaces and within the demineralized dentin. As the reactions and the deposition of Ca-P complexes continue, this layer crystallizes into hydroxycarbonate apatite, which is chemically and structurally similar to biological apatite.[22,23] The combination of the residual calcium sodium phosphosilicate particles and the HCA layer results in remineralization and physical occlusion of dentinal tubules. The chemical reactions initiated by calcium sodium phosphosilicate to promote the formation of an HCA might also be useful in treating demineralized tooth structure, preventing further demineralization and remineralizing the hybrid layer within the resin–dentin interface when used during bonding procedures. However, although Nupro NU Solutions™ is the only prophy paste containing bioactive glass doped with calcium and phosphate ions, this study has demonstrated that a single application and 24 or 48 hours of immersion in RSS solution is not sufficient for this product to induce dentin remineralization.

In conclusion, we have to reject the null hypothesis that all the dental materials used in this study are able to remineralize the dentin and occlude the dentinal tubules after immersion in a remineralizing saline solution (RSS) for 24 or 48 hours.

Further studies are in progress to evaluate the remineralization effects of the bioactive glass on the hybrid layers when used during air-cutting or bonding procedures to increase the longevity of the restorations.

ACKNOWLEDGMENT

This study was supported by the 2010 Ralph Phillips Research Fund of the Academy of Operative Dentistry. The authors also acknowledge support from the Department of Health via the National Institute for Health Research (NIHR) Invention for Innovation (i4i) Programme award to King's College London' Trust.

(Accepted 6 December 2010)

REFERENCES

1. Brännström M, Linden LA, & Johnson G (1968) Movement of dentinal and pulpal fluid caused by clinical procedures *Journal of Dental Research* **47(5)** 679-682.
2. Addy M & Pearce N (1994) Aetiological, predisposing and environmental factors in dentine hypersensitivity *Archives of Oral Biology* **39(Supplement)** 33S-38S.
3. Pashley DH, Mattews WG, Zhang Y, & Johnson M (1996) Fluid shifts across human dentin in vitro in response to hydrodynamic stimuli *Archives of Oral Biology* **41(11)** 1065-1072.
4. Addy M, Absi EG, & Adams D (1987) Dentine hypersensitivity: The effects in vitro of acids and dietary substances on root-planed and burred dentine *Journal of Clinical Periodontology* **14(5)** 274-279.
5. Lussi A, Kohler N, Zero D, Schaffner M, & Megert B (2000) A comparison of the erosive potential of different beverages in primary and permanent teeth using an in vitro model *European Journal of Oral Science* **108(2)** 110-114.
6. Clark C, Woo G, Silver JG, Sweet D, & Grisdale JC (1990) The influence of frequent ingestion of acids in the diet on treatment for dentin sensitivity *Journal of the Canadian Dental Association* **56(12)** 1101-1103.
7. Absi EG, Addy M, & Adams D (1992) Dentine hypersensitivity—The effect of toothbrushing and dietary compounds on dentine in vitro: A SEM study *Journal of Oral Rehabilitation* **19(2)** 101-110.
8. West NX, Hughes JA, & Addy M (2001) The effect of pH on the erosion of dentine and enamel by dietary acids in vitro *Journal of Oral Rehabilitation* **28(9)** 860-864.
9. Prati C, Montebugnoli L, Suppa P, Valdre G, & Mongiorgi R (2003) Permeability and morphology of dentin after erosion induced by acidic drinks *Journal of Periodontology* **74(4)** 428-436.
10. Litkowski LJ, Hack GD, Sheaffer HB, & Greenspan DC (1997) Occlusion of dentin tubules by 45S5 Bioglass. In: Bioceramics Vol. 10 Proceedings of the 10th International Symposium on Ceramics in Medicine *Elsevier Scientific* 411-414.
11. Forsback AP, Areva S, & Salonen JI (2004) Mineralization of dentin induced by treatment with bioactive glass S53P4 in vitro *Acta Odontologica Scandinavica* **62(1)** 14-20.
12. Vollenweider M, Brunner TJ, Knecht S, Grass RN, Zehnder M, Imfeld T, & Stark WJ (2007) Remineralization of human dentin using ultrafine bioactive glass particles *Acta Biomaterials* **3(6)** 936-943.
13. Sauro S, Watson TF, Mannocci F, Tay FR, & Pashley DH (2009) Prevention of water contamination of ethanol-saturated dentin and hydrophobic hybrid layers *Journal of Adhesive Dentistry* **11(4)** 271-8.
14. Pashley DH (1996) Dynamics of the pulpo-dentin complex *Critical Reviews in Oral Biology and Medicine* **7(2)** 104-133.
15. Sauro S, Watson TF, & Thompson I (2010) Dentine desensitization induced by prophylactic and air-polishing procedures: an in vitro dentine permeability and confocal microscopy study *Journal of Dentistry* **38(5)** 411-422.
16. Pashley DH & Depew DD (1986) Effects of smear layer, copalite and oxalate on microleakage *Operative Dentistry* **11(3)** 95-102.
17. Hench LL & Andersson Ö (1993) Bioactive glasses. In: Hench LL, Wilson J (eds) *Introduction to Bioceramics* World Scientific, Singapore 45-47.
18. Paolinelis G, Banerjee A, & Watson TF (2008) An in vitro investigation of the effect and retention of bioactive glass air-abrasive on sound and carious dentine *Journal of Dentistry* **36(3)** 214-218.
19. Tsuda H, Ruben J, & Arends J (1996) Raman spectra of human dentin mineral *European Journal of Oral Science* **104(2, Part 1)** 123-131.
20. Tsuda H & Arends J (1997) Raman spectroscopy in dental research: A short review of recent studies *Advances in Dental Research* **1(4)** 539-547.
21. Gandolfi MG, Silvia F, H PD, Gasparotto G, & Carlo P (2008) Calcium silicate coating derived from Portland cement as treatment for hypersensitive dentine *Journal of Dentistry* **36(8)** 565-578.
22. Andersson OH & Kangasniemi I (1991) Calcium phosphate formation at the surface of bioactive glass in vitro *Journal of Biomedical Materials Research* **25(8)** 1019-1030.
23. Hench LL & Andersson Ö (1993) Bioactive glasses. In: Hench LL, Wilson J (eds) *Introduction to Bioceramics* World Scientific, Singapore 45-47.
24. Cerruti MG, Greenspan D, & Powers K (2005) An analytical model for the dissolution of different particle size samples of Bioglass in TRIS buffered solution *Biomaterials* **26(24)** 4903-4911.