Revista Portuguesa de Estomatologia, Medicina Dentária e Cirurgia Maxilofacial

REV PORT ESTOMATOL MED DENT CIR MAXILOFAC. 2017;58(1):8-16

Original research

Ultramorphological and chemical characterization of dentin surfaces after application of two desensitizing toothpastes

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ARTICLE INFO

Article history:
Received 5 August 2016
Accepted 24 November 2016
Available online 30 March 2017

Keywords:
Dentin hypersensitivity
Dentin tubules
Energy dispersive X-ray
Scanning electron microscopy
Toothpaste

ABSTRACT

Objectives: To evaluate dentin tubule obliteration after application of two different desensitizing toothpastes using scanning electron microscopy (SEM) for ultramorphological analysis and energy dispersive X-ray spectroscopy (EDX) for chemical evaluation.

Methods: Five dentin discs were sectioned into four quarters; surfaces were etched with 6% citric acid for 2 minutes and equally distributed into four groups. In G1 (control) no treatment was performed; dentin surfaces were brushed twice-daily during 14 days with artificial saliva (G2), a combined stabilized stannous fluoride, sodium hexametaphosphate and silica (SFSH) toothpaste (G3) and a calcium sodium phosphosilicate (CSFS) toothpaste (G4), under a standardized protocol. All specimens were analyzed by SEM and EDX and tubule occlusion was scored. Statistical analysis was performed using Kruskal-Wallis ($p<0.05$).

Results: Score distribution across groups consistently increased from G1 to G4, being the last the most consistent group. Statistical between-treatment comparisons for 750-fold and 2000-fold magnification revealed significant differences between groups ($p=0.009$ and $p=0.002$, respectively). For both magnifications, post-hoc analysis adjusted for multiple comparisons only indicated statistically significant differences between G1 and G4 ($p=0.012$ and $p=0.001$, respectively). Chemical analysis revealed high levels of carbon, oxygen and nitrogen for G1. For G2 an increase of the levels of the phosphorous and calcium elements and a drop of oxygen and carbon levels was registered. G3 and G4 showed a surface layer mainly composed of calcium and phosphorous.

Conclusions: Both desensitizing toothpastes induced high levels of tubule occlusion with consistent phosphorus and calcium deposition over dentin surface.

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http://doi.org/10.24873/j.rpemd.2017.05.004

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Caracterização ultramorfológica e química de superfícies dentinárias após aplicação de duas pastas dentífricas dessensibilizantes

Palavras-chave:
Hipersensibilidade dentária
Túbulos dentários
Raios X por dispersão em energia
Microscopia electrónica de varrimento
Pasta dentífrica

R E S U M O

Objectivos: Avaliar a obliteração dos túbulos dentinários após a aplicação de duas pastas dentífricas dessensibilizantes, usando microscopia eletrónica de varrimento (MEV) e espectroscopia de raios X por dispersão em energia (EDX).

Métodos: Foram seccionados cinco discos de dentina em quatro quartos, condicionados com ácido cítrico a 6% por 2 minutos e distribuídos equitativamente em quatro grupos: No G1 (controlo) não se efetuou qualquer tratamento; as superfícies dentinárias foram escovadas duas vezes por dia durante 14 dias com saliva artificial (G2), uma pasta de fluoreto estanhoso estabilizado, hexametafosfato de sódio e sílica (SFSH) (G3) e uma pasta de fosfosilicato de sódio e cálcio (CSFS) (G4). As amostras de cada grupo foram quantificadas por MEV e EDX. Na análise estatística aplicou-se o teste de Kruskal-Wallis ($p<0,05$).

Resultados: O grau de oclusão tubular aumentou de G1 até G4, sendo este último o mais consistente.Para as ampliações de 750x e 2000x foram encontradas diferenças estatisticamente significativas entre grupos ($p=0,009$ e $p=0,002$, respetivamente). Em ambos os casos a análise post-hoc ajustada para comparações múltiplas apenas identificou diferenças entre G1 e G4 ($p=0,012$ e $p=0,001$, respetivamente). A análise química revelou elevados níveis de carbono, oxigénio e nitrogénio no G1. Para o G2 registou-se um aumento dos níveis de fósforo e cálcio com uma diminuição concomitante de oxigénio e carbono. Os G3 e G4 apresentavam maioritariamente fósforo e cálcio.

Conclusões: Ambas as pastas dessensibilizantes induziram um elevado grau de oclusão tubular com uma deposição consistente de fósforo e cálcio sobre a superfície dentinária.

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Introduction

Dentin hypersensitivity (DH) is defined as a short, sharp pain arising from exposed dentin in response to typically thermal, evaporative, tactile, osmotic or chemical stimuli.[1] DH-related discomfort may have a significant negative impact on an individual's daily life, as it may cause difficulties in eating, drinking and speaking.[2] Due to its high prevalence, significant efforts have been made to understand the etiology and mechanisms involved in DH development.[3] Several conditions were identified, among them: gingival recession; periodontal disease; deep tooth cracks and loss of enamel, cementum, and dentin due to mechanical abrasion, chemical erosion, and tooth fracture.[4,5]
A common feature of DH is the presence of open dentin tubules, which provide a direct link between the external environment and the tooth pulp.[6] There are a large number of options for managing DH using chemical or physical agents. Current treatments tend to concentrate on two approaches: neural transmission blockage or tubule occlusion.[7] Recently, two new promising molecules were developed for hypersensitivity management: stabilized stannous fluoride containing sodium hexametaphosphate (SFSH) and a calcium sodium phosphosilicate (CSPS).

Stannous fluoride has been incorporated in dental dentifrices due to its therapeutic behavior in different fields, such as protection against carious pathogenic bacteria, gingivitis, hypersensitivity and plaque development.[8] However, its clinical usage was limited because of astringent taste and extrinsic staining of the teeth. Those limitations were outdated when a novel dentifrice introduced a new formulation combining stabilized stannous fluoride, sodium hexametaphosphate, and silica (SFSH). This formula offers the therapeutic benefits of a 0.454% stabilized stannous fluoride and stain-control characteristics of sodium hexametaphosphate in a low-water formulation dentifrice.[9] When this anhydrous preparation is applied on dentin surfaces the occlusion of tubules by a tin-rich low solubility complexes is expected.[10]

Calcium sodium phosphosilicate (CSPS) is an inorganic amorphous compound that contains calcium, sodium, phosphate and silica.[11] When CSPS particles contact an aqueous environment, an immediate release of sodium ions occurs, which increases local pH environment. The surface reaction include the ion exchange between Na^{2+} from CSPS and H^{+} from dentin fluid resulting in the formation of a porous silica rich layer on the surface, that provides a nucleating site for early precipitation of a calcium phosphate hydroxycarbonate apatite layer.[12]

The aim of this *in vitro* study was to evaluate the effectiveness of two desensitizing dentifrices, SFSH- and CSPS-based, in occluding dentinal tubules using scanning electron microscopy

(SEM). The null hypothesis is that there are no differences regarding dentin tubule occlusion between the materials tested.

Material and methods

Five caries free human third molars were collected after obtaining patient informed consents, as approved by the Ethical Committee. The teeth were cleaned and stored at room temperature in a 10% buffered formalin solution (pH 7.0). Five dentin discs of 1 mm thickness were obtained by sectioning each tooth parallel to the occlusal surface from the top of the pulp horns and occlusally using a hard tissue cutting saw (Accutom 50, Struers, Ballerup, Denmark), with water as coolant. Each disc was then sectioned into four quarters that have been identified and singly stored in artificial saliva until required (Figure 1).

All specimens had their smear layer removed by ultrasonication in deionized water followed by surface etching with 6% citric acid for 2 minutes and rinsing with distilled water for 30 seconds to create opened dentin tubules. The specimens from each tooth were equally distributed into four groups, each containing five samples (n=5). In Group 1 (control), samples were immersed in artificial saliva for 14 days. For the other groups, a single-tuft toothbrush mounted in an electric brushing device (Oral-B® Professional Care® 500, Procter & Gamble Co., Cincinnati, OH, USA) was applied perpendicular to the dentin surface (Figure 1), at a constant loading for 30 seconds, twice daily (12 hours interval) for 14 days. In G2, samples were brushed with 4 ml of artificial saliva, whilst in G3 and G4 specimens were brushed with 40 g of the respective toothpaste, SFSH (Oral-B® Pro Expert; Procter & Gamble UK, Weybridge, UK) and CSPS (Sensodyne® Repair&Protect; GlaxoSmithKline; Slough, UK), without any dilution. After brushing, samples were gently rinsed with 10 ml of deionized water for 10 seconds, and stored in artificial saliva at 37.°C, until used in the next brushing session. Artificial saliva was changed between all brushing periods. (Table 1)

For SEM analysis, the samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer for 48h at room temperature. The specimens were then dehydrated in ascendant alcohol solutions (50%, 75% 90%, 95%, 100%) and submitted to chemical drying in hexamethyldisilazane. All samples were mounted on aluminum stubs using carbon sticky pads, sputter coated with gold and subjected to SEM analysis (JSM 5310, JEOL; Tokyo, Japan). The acceleration voltage was set at 10 kV. To achieve a cross section view, samples were then fractured into halves. To assess the level of tubule occlusion, photomicrographs were taken from each dentin surface at a 750-fold and 2000-fold magnification. Image grading was performed based on those micrographs, by two blinded evaluators once and independently. When disagreements arose, the examiners had to reach a consensus. Evaluation was undertaken according to a six-point scale:

- Score 1 (Sc1): open tubules;
- Score 2 (Sc2): most tubules open (~90%);
- Score 3 (Sc3): half of tubules occluded (~50%);
- Score 4 (Sc4): most tubules occluded but tubules outlines visible;
- Score 5 (Sc5): most tubules occluded (~90%);
- Score 6 (Sc6): all tubules occluded.

Additionally, energy dispersive X-ray (EDX) analysis was performed from two surface samples of each group. The acceleration voltage of the scanning electron microscope was set to 20 kV and EDX spectra were collected using a Si-detector (X-Max^{N} detector, Oxford Instruments, Oxfordshire, UK). Spectra were processed using AZtecEnergy analysis software (AZtec, Oxford Instruments, Oxfordshire, UK) for surface element composition detection and for relative element contents calculation in weight percentage.

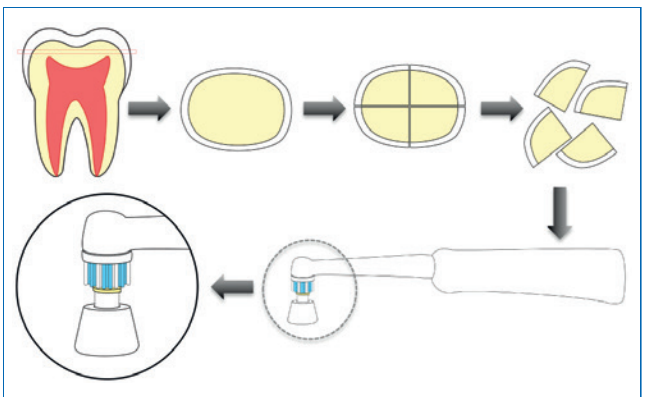

Figure 1. Schematic diagram of specimen preparation form a single tooth and brushing procedures.

Table 1. Artificial saliva and toothpastes composition.

Materials	Composition (mg/1,000 ml)	Lot nr
G2 Artificial saliva	Nacl 125.6,KC 963.9,CaCl2.2H2O 227.8, KH2PO4 654.5,Urea 200.0, NH4Cl 178.0, NaHCO3 630.8, KSCN 189.2, Na2SO4.10H2O 763.2	
G3 SFSH Oral-B® Pro Expert Procter & Gamble UK, Weybridge, UK	Glycerin, Hydrated Silica, Sodium Hexametaphosphate, Propylene Glycol, PEG-6, Aqua, Zinc Lactate, Sodium Lauryl Sulfate, Aroma, Sodium Gluconate, Chondrus Crispus Powder, Trisodium Phosphate, Stannous Fluoride, Sodium Saccharin, Xanthan Gum, Copernicia Cerfera Cera, Cinnamal, Silica, Sodium Fluoride, cl 77891, Eugenol, cl 74160. Fluor(1450ppm)	GGC8
G4 CSPS Sensodyne® Repair&Protect (GlaxoSmithKline; Slough, UK)	Glycerin, Silica, Calcium Sodium Phosphosilicate (NovaMin), Sodium Lauryl Sulfate, Sodium Monofluorophosphate, Aroma, Titanium Dioxide, Carbomer, Potassium Acesulfame, Limonete, Fluor (1450ppm).	152D G1

Statistical analysis was performed using IBM® SPSS Statistics Version 20.0 (SPSS, Chicago, IL, USA). Non-parametric group comparison was performed using Kruskal-Wallis and all pairwise as post-hoc comparisons. Wilcoxon signed-ranks test was applied for intragroup comparison at 750-fold and 2000-fold magnifications. Significance level was set at 0.05.

Results

The between-observers agreement was quantified by intra-class correlation coefficient analysis for single measures that showed a high level of agreement (ICC= 0.937, $p<0.01$).

Statistical between-treatment comparisons of the occlusion scores mean ranks for 750-fold and 2000-fold magnification revealed significant differences between groups (p=0.009 and p=0.002, respectively). For 750-fold and 2000-fold magnification, post-hoc analysis adjusted for multiple comparisons only indicated statistically significant differences between G1 and G4 (p=0.012 and p=0.001, respectively). However, for both magnifications, G1 showed the lowest mean score indicating the least percentage of tubule occlusion while G4 showed the maximum mean score, with the highest level of occlusion and the most consistent results in the final score distribution (Figure 2)

Wilcoxon signed ranks test showed no differences between scores obtained at the 750-fold and the 2000-fold magnification (Z= -0.087, p=0.931).

All samples of G1 showed open dentin tubules (Figures 3-6). In G2, samples showed a reduction in the tubule lumen diameter and a considerable number of obliterated tubules, while maintaining tubules outlining remained mostly visible. Tubule entrance was occasionally filled with precipitates with no more than a 2 μm depth (Figures 7-10). G3 dentin surfaces presents an incomplete surface coating layer where a great number of dentinal tubules became partially or completely obliterated and precipitates occlude tubule lumens in an inhomogeneous form (Figures 11-14). In G4, most surfaces appeared completely covered by an irregular layer with few or no open tubules discernible occluded with precipitates (Figures 15-18).

According to the EDX analysis, relative element contents calculation in weight percentage (wt.%) obtained for each group are shown in Table 2. G1 showed high levels of carbon, oxygen, and nitrogen. For G2, chemical mapping showed an increase of the levels of the phosphorous and calcium elements and a simultaneous drop of carbon levels. For G3, EDX showed that surface layer was mainly composed of calcium and phosphorous. Additionally, signs of silicon, zinc and sodium were also found. For G4 EDX showed that occlusion deposits were predominantly composed of calcium and phosphorous. Additionally, discrete signs of titanium and sodium were also found.

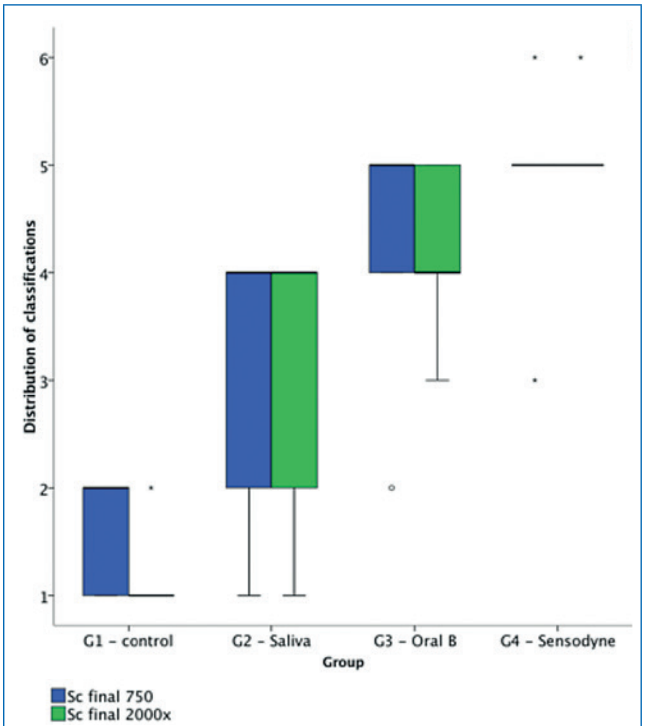

Figure 2. Score distribution per group, for 750x and 2000x magnifications.

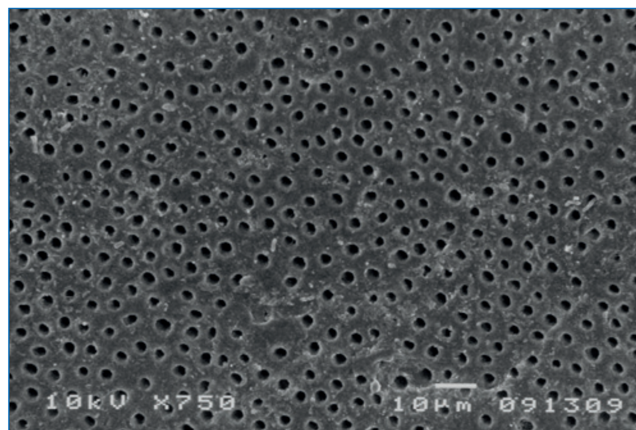

Figure 3. Representative SEM photograph of a dentin surface immersed in artificial saliva (G1) observed at a 750-fold magnification. Mainly open orifices of dentin tubules are shown.

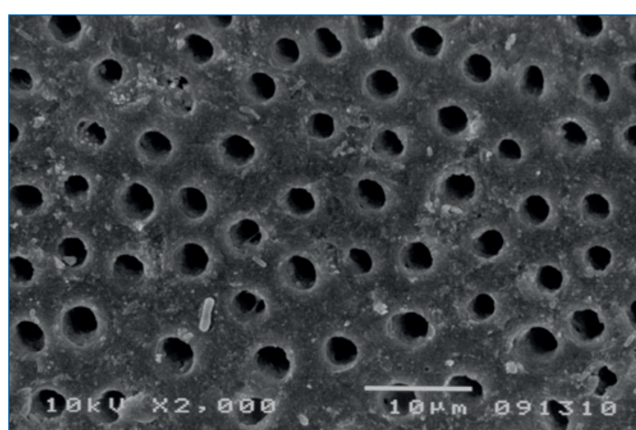

Figure 4. Representative SEM photograph of a dentin surface immersed in artificial saliva (G1) observed at a 2000-fold magnification.

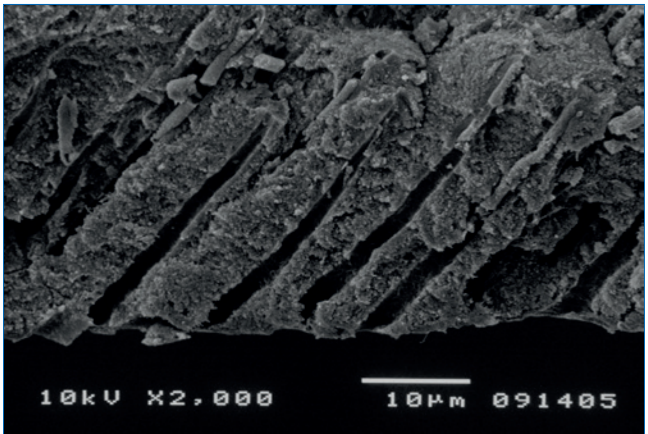

Figure 5. **Representative SEM photograph of a cross-sectioned dentin sample immersed in artificial saliva (G1) observed at a 2000-fold magnification. Open tubules running from the surface are evident reaching a 20µm demineralization depth.**

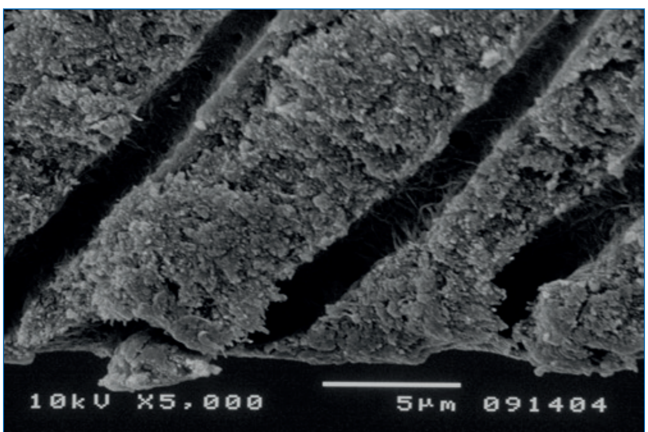

Figure 6. **Representative SEM photograph of a cross-sectioned dentin sample immersed in artificial saliva (G1) observed at a 5000-fold magnification.**

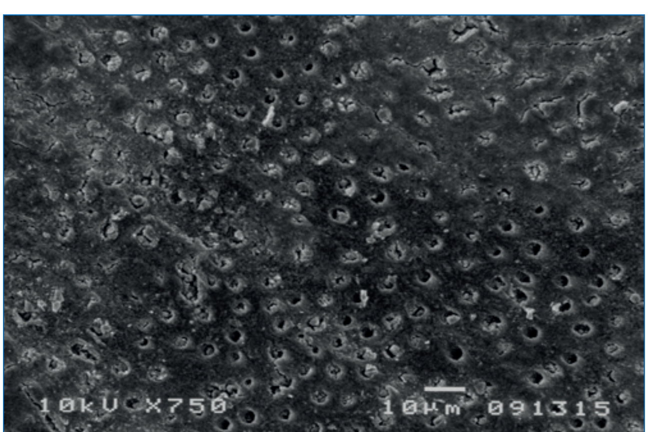

Figure 7. **Representative SEM photograph of a dentin surface brushed with artificial saliva (G2) observed at a 750-fold magnification. Reduced lumen diameter size with several obliterated dentin tubules are shown, while maintaining tubules outlines visible.**

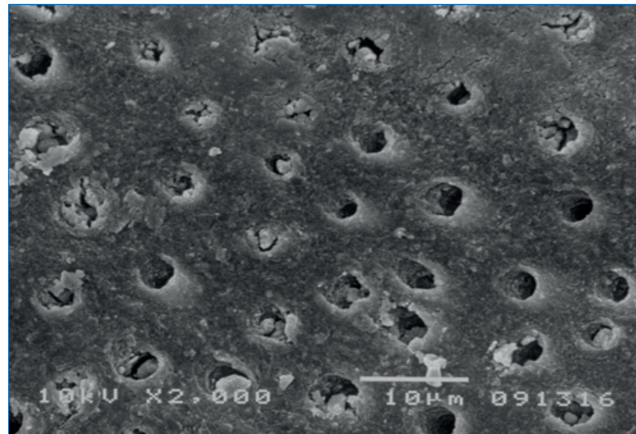

Figure 8. **Representative SEM photograph of a dentin surface brushed with artificial saliva (G2) observed at a 2000-fold magnification.**

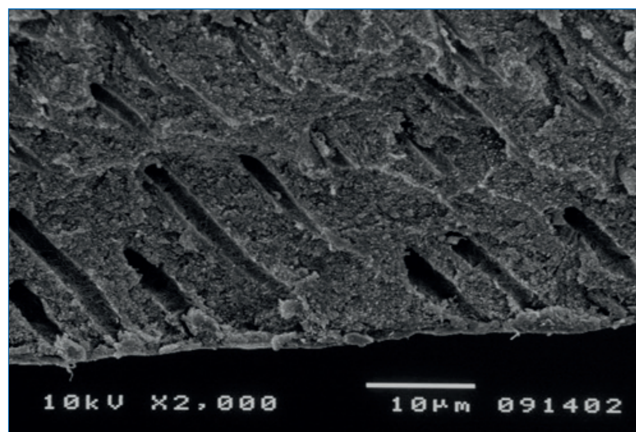

Figure 9. **Representative SEM photograph of a cross-sectioned dentin sample brushed with artificial saliva (G2) observed at a 2000-fold magnification. Discrete precipitations can be observed inside the entrance of dentin tubules.**

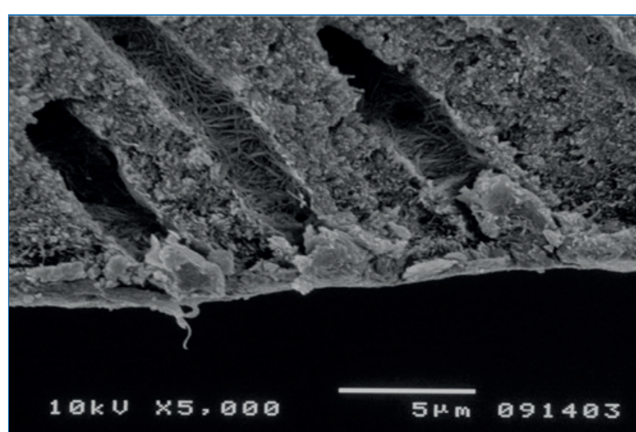

Figure 10. **Representative SEM photograph of a cross-sectioned dentin sample brushed with artificial saliva (G2) observed at a 5000-fold magnification.**

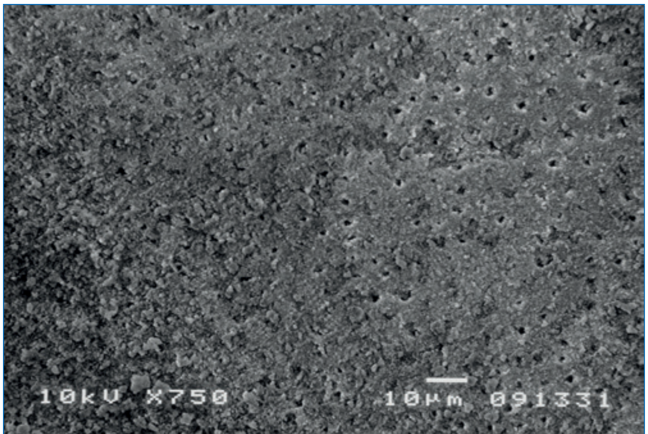

Figure 11. Representative SEM photograph of a dentin surface brushed with SFSH (G3) observed at a 750-fold magnification. It can be perceived an incomplete coating layer covering partially or completely tubule lumen.

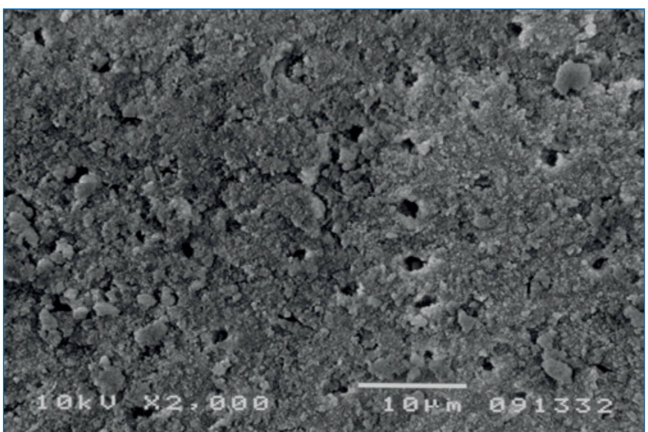

Figure 12. Representative SEM photograph of a dentin surface brushed with SFSH (G3) observed at a 2000-fold magnification.

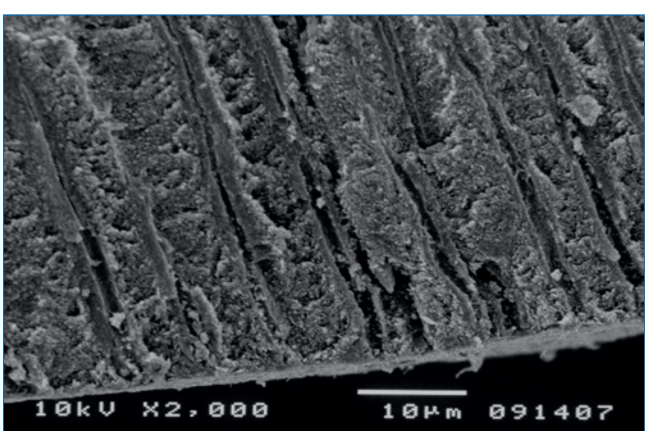

Figure 13. Representative SEM photograph of a cross-sectioned dentin sample brushed with SFSH (G3) observed at a 2000-fold magnification. It can be observed a generalized reduction in tubule diameter and precipitates occluding tubule lumen.

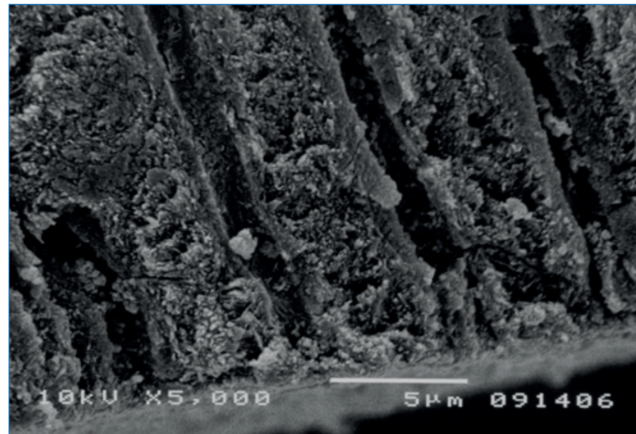

Figure 14. Representative SEM photograph of a cross-sectioned dentin sample brushed with SFSH (G3) observed at a 5000-fold magnification.

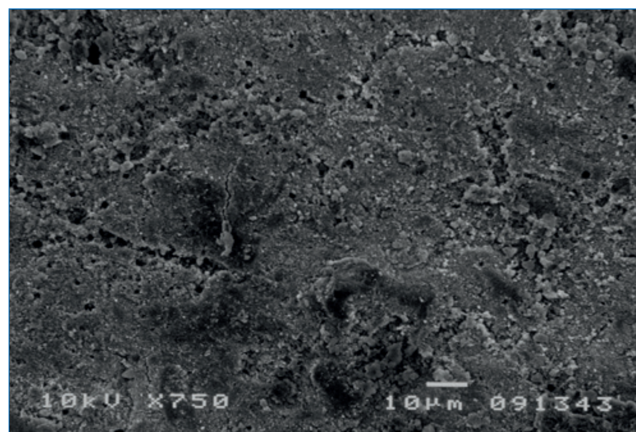

Figure 15. Representative SEM photograph of a dentin surface brushed with CSPS (G4) observed at a 750-fold magnification. It can be perceived a irregular covering layer with a few or no open tubules discernible.

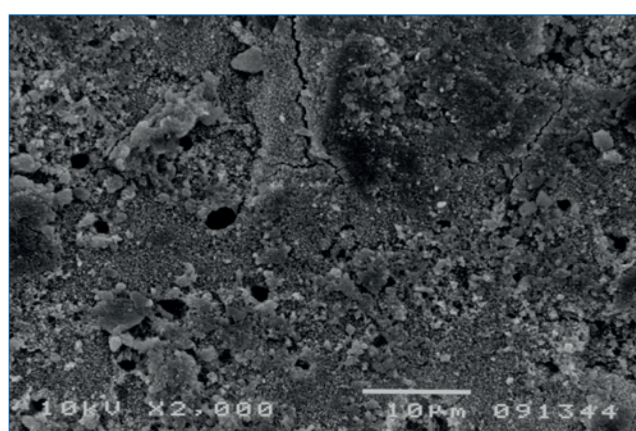

Figure 16. Representative SEM photograph of a dentin surface brushed with CSPS (G4) observed at a 2000-fold magnification.

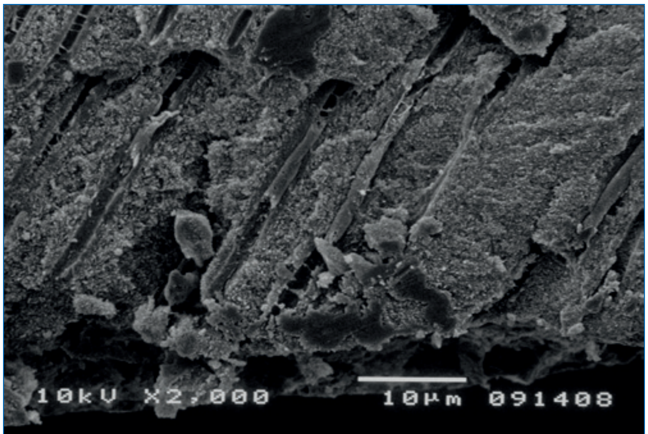

Figure 17. Representative SEM photograph of a cross-sectioned dentin sample brushed with CSPS (G4) observed at a 2000-fold magnification. Significant reduction or even looseness of tubule lumens are evident with precipitates covering the surface.

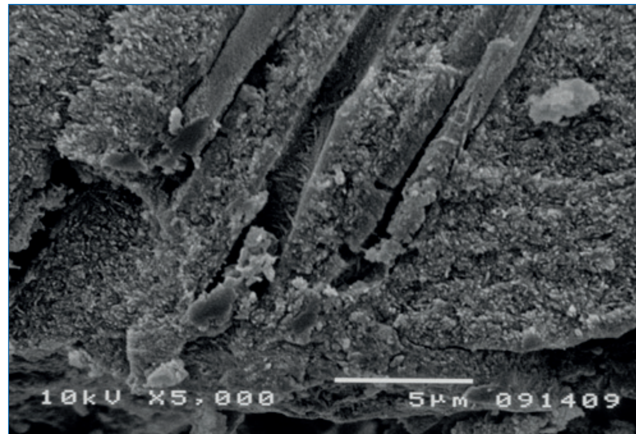

Figure 18. Representative SEM photograph of a cross-sectioned dentin sample brushed with CSPS (G4) observed at a 5000-fold magnification.

Table 2. Relative element contents calculation in weight percentage (wt.%) for each group obtained from EDX analysis.

Spectrum Labe	C	N	O	Na	Si	P	Ca	Ti	Zn	Total
G1 – control	83.29	7.49	9.08			0.03	0.12			100.0
G2 – Artificial saliva	16.09		44.75			9.04	30.12			100.0
G3 – SFSH	6.97		19.09	0.71	4.14	14.38	51.92		2.80	100.0
G4 – CSPS	11.59		30.94	0.84		17.64	38.33	0.66		100.0

(C: carbon; N: nitrogen; O: oxygen; Na: sodium; Si: silicon; P: phosphorous; Ca: calcium; Ti: Titanium; Zn: zinc)

Discussion

Dentin hypersensitivity is considered one of the most prevalent painful condition of the oral cavity, but it is poorly understood. The ideal treatment has not yet been reached and no gold standard treatment has been advocated.[13,14]

The dentin disc model has been used in several earlier studies and was considered to represent a close approximation of the *in vivo* situation.[15] In order to improve this model, homogenization of dentin substrate across groups was ensured, as dentin substrates can exhibit different features from tooth to tooth.[16-18]

SEM analyses were performed using 750-fold and 2000-fold magnifications as reported in other studies.[19-22] Nevertheless, the use of a 750-fold magnification can be considered more reliable for precise evaluation, as a relatively broad image is available for accurate quantification.

In some instances, natural occlusion of tubules occur due to smear layer formation or calcium phosphate deposits mainly derived from saliva.[23] However, these occlusions may be easily modified by tooth brushing or acid challenge, dislodging the tubule obstruction. This may explain why the DH condition is related to frequent episodes of acute pain followed by periods of quiescence.[23] In fact, G2 showed a considerable number of obliterated tubules, which can be attributed to calcium phosphate precipitation on the dentin surface, because artificial saliva is supersaturated with respect to hydroxyapatite.[24]

Both toothpastes induced a well-developed occluding ability. Therefore, the null hypothesis could not be rejected. Nevertheless, the occlusion pattern tended to be higher and more homogeneous for the CSPS.

Quantitative SEM score results and chemical analyses for CSPS toothpaste obtained in the present study was similar to those reported by others authors.[25-28] The mineralized surface layer detected in treated samples was previously described as a mixture of nano-crystalline and amorphous material, composed by a hydroxyapatite-like residue, resulting in the formation of a calcium-phosphate enriched layer resistant to acid and mechanical challenges.[25,26,29] This stabilized surface layer can result in tubular occlusion, but also in the potential chemical interaction of CSPS with exposed type I collagen fibers.[30] Accordingly, the EDX surface map evidenced the main presence of Ca and P, as expected. Low levels of Ti and Na were also detected and the Ti signal is thought to result from TiO_2 formation.[25] These results are consistent with clinical findings reported in the literature indicating that CSPS is an effective agent for reducing tooth sensitivity assessed by randomized controlled clinical trials and in a recent meta-analysis.[31-36]

Concerning the SFSH dentifrice, few studies were published. An experimental study showed that when a stannous fluoride anhydrous preparation was brushed in dentin, a nearly complete coverage of the dentine surface and occlusion of tubules by a tin-rich surface deposit was observed.[10] In the present study, high contents of Ca and P were found along with small levels of Si, Zn and Na. The Si and Zn signals should have resulted from SiO_2 and ZnO_2 formation.[24] Surprisingly, the tin element was not identified in spectra, although a discrete peak at the typical tin-specific L_α line at 3.443 keV has been detected.[37] Similar results were obtained by Ganss et al.[38], whose work showed that the amount of tin retained in sound dentin and on surfaces where the organic matrix was preserved was much lower than on dentin surfaces that underwent severe erosive conditions. Besides, they reported a considerably thick continuous layer covering sound dentin surface, consisting mainly on Ca and P and relatively small amounts of tin, emphasizing that the mechanism behind this covering is unclear. No accurate conclusions can be drawn on the source of the Ca, P and O signals, but it may be related to a slight demineralization of organic matrix.[40] It is possible that the tin signal could be found more deeply in dentin, as tin uptake is related to a dose-dependent diffusion control deep through the collagen structure. The chemical interaction with tin can occur either with the mineral content by the formation of tin salts and/or with collagen or other dentin protein that contains negatively charged groups, capable of binding cations with high affinity.[38] Therefore, the occlusion of tubule lumens that became evident after brushing treatment with SFSH toothpaste, could be due to both pathways. Several *in vivo* reports showed that dentifrices or gels containing stannous fluorides had a significant effect in reducing sensitivity in the long-term.[39-42]

In order to improve the experimental methodology, further studies should be performed with an increase in sample size using lower magnifications for evaluation, while subjecting samples to daily erosive or mechanical challenges. These features would allow a more accurate simulation of real clinical conditions.

Conclusions

According to the present *in vitro* study no statistically significant differences on the occlusion ability of dentin tubules were found between SFSH and CSPS toothpastes. However, a more homogenous dentin occlusion was achieved with CSPS dentifrice. Brushing with artificial saliva produced a more limited dentin tubule occlusion when compared to the toothpastes.

From EDX analysis, brushing either with SFSH or CSPS conduces to high levels of phosphorus and calcium deposition over dentin tubules.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgements

The authors would like to express their gratitude to Dr Francisco Basto and Eng António Fonseca for their technical support.

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