

Effect of desensitizing toothpastes on dentin

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Abstract: The objective of this study was to analyze the effects of toothbrushing with desensitizing toothpastes on dentin permeability and dentinal tubule occlusion. Fifty rats provided two hundred incisor teeth divided into five groups: DW, brushed with distilled water (control); FT, brushed with fluoride toothpaste; SCT, brushed with strontium chloride toothpaste; PCT, brushed with potassium citrate toothpaste; and PNT, brushed with potassium nitrate toothpaste. Cavities were prepared to expose the dentinal tubules, and the incisor teeth were brushed using the experimental agents. After each treatment, Evans blue dye solution was applied to the teeth. Dentin permeability was analyzed using scanning electron microscopy and energy-dispersive X-rays (EDX). There were significant differences ($p < 0.0001$, ANOVA) among the groups regarding dentin permeability, number of dentinal tubules, diameter of dentinal tubules, and opened tubular area. In the SCT, PCT and PNT groups, opened and partially occluded tubules, deposits, and a few smear layers were observed. In the DW and FT groups, most of the dentinal tubules were open, with no deposits or smear layers on the dentin. EDX revealed peaks of calcium and phosphorus in all of the groups, as well as traces of strontium in the SCT group and of potassium in the PCT and PNT groups. Desensitizing toothpaste decreased dentin permeability, although it produced only partial dentin tubule occlusion.

Descriptors: Dentin Desensitizing Agents; Toothpastes; Toothbrushing.

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Introduction

The improvement of oral health is necessary for the reduction of caries and for long-term tooth maintenance. However, oral hygiene procedures can also contribute to tooth wear, mainly when they are performed in areas with exposed dentin. Dentin hypersensitivity (DH) is neither a recent problem nor a rare one; nonetheless, this clinical condition remains poorly understood with no effective or permanent treatment available.^{1,2}

DH is clinically described as a painful response to thermal, chemical, mechanical, evaporative, or osmotic stimuli applied to opened dentinal tubules, which cannot be ascribed to any other form of dental defect or pathology.^{1,3}

The dentinal tubules play an important role in transferring stimuli and irritants to the pulp. The hydrodynamic theory of dentin sensitivity postulates a flow of fluid through the tubule as the transducing mechanism for hydrodynamic stimuli.²⁻⁴ It has been demonstrated that hyper-

sensitive dentin has a larger number of wide, opened dentinal tubules on the surface than does non-sensitive dentin.⁵ A rational approach to the control of pain arising from exposed dentin would thus be to block the tubules or to reduce their diameter.⁵⁻⁸

Ideally, a treatment for dentin hypersensitivity, as well as for other problems, should be quick and simple. Thus, the use of special toothpastes has been largely embraced as one of the first alternatives. They are non-invasive and easily available (in drug-stores and supermarkets), and according to some studies, they have a good cost-benefit ratio, especially compared to professional appointments.^{1,2,4,5,8-11}

Dentinal tubule occlusion in humans, at present, can only be evaluated by extraction, replication or dentin biopsy, all of which have their own problems and limitations. Animal models provide a better option for studying the action of desensitizing agents on dentin. Thus, the purpose of this study was to compare the effects of desensitizing toothpastes, in combination with toothbrushing, on dentin permeability and on dentinal tubule occlusion in rats.

Methodology

Fifty rats provided two hundred teeth (upper and lower incisors) for the study (Research Ethics Committee: #629006). The animals were divided into five groups (n = 10 rats *per* group):

- DW, brushed with distilled water (control);
- FT, brushed with 1500 ppm fluoride toothpaste;
- SCT, brushed with 10% strontium chloride toothpaste;
- PCT, brushed with 5.5% potassium citrate

toothpaste; and

- PNT, brushed with 5.0% potassium nitrate toothpaste.

The toothpaste compositions are summarized in Table 1.

The animals were anesthetized with an intraperitoneal injection (ketamine 75 mg/kg and xylazine 10 mg/kg). Cavities 0.3 mm in depth and 0.8 mm in width were prepared on the buccal surfaces (cervical region) of the upper and lower incisors (forty teeth *per* group) using a standard (active tip with 0.3 mm) #245 carbide burr (S.S. White, Rio de Janeiro, Brazil).⁷ Prior to the treatments, the dentin of all of the cavities was treated with 24% EDTA gel (Biodinâmica Química e Farmacêutica, Ibiporã, Brazil) on tiny cotton pellets, which were replaced every 30 s for 3 min to remove the smear layer and to open the dentinal tubules.¹²

The teeth were brushed with a children's toothbrush (Colgate Kids®, São Paulo, Brazil) using the experimental agents (tests: 2 g of the toothpaste with 1 mL of distilled water; control: 2 mL of distilled water). This procedure was performed once daily for 4 days. After each daily treatment, 5 µL of 5% Evans blue dye solution (Merck Chemical, Darmstadt, Germany) was applied with a dropping pipette onto each dentin cavity and was left to penetrate for 5 min.

Fifteen toothbrushing movements were applied, based on clinical aspects, because fifteen is the normal number of movements performed during toothbrushing *per* tooth. The force for toothbrushing was

Table 1 - Toothpastes used in the study.

Group	Toothpaste	Active ingredients	Company
FT	Colgate Tripla Ação®	Sodium monofluorophosphate (1500 ppm fluoride), calcium carbonate, sodium silicate, sodium bicarbonate	Colgate-Palmolive, São Paulo, Brazil
SCT	Sensodyne Original®	10% strontium chloride hexahydrate, silica, titanium dioxide, calcium carbonate	GlaxoSmithKline, Rio de Janeiro, Brazil
PCT	Colgate Sensitive Branqueador®	5.04% potassium citrate, sodium monofluoro-phosphate (1450 ppm fluoride), silica, titanium dioxide	Colgate-Palmolive, São Paulo, Brazil
PNT	Sensodyne Proteção Total®	5.0% potassium nitrate, triclosan, silica, titanium dioxide, calcium carbonate	GlaxoSmithKline, Rio de Janeiro, Brazil

applied by the same examiner, and this force was calibrated.

At the end of experiment, the rats were sacrificed, and the teeth were carefully extracted.

Dentin permeability analysis

Five percent by weight Evans blue solution was employed to analyze the dentin permeability (20 teeth *per* group). The samples were embedded in epoxy resin and were sectioned longitudinally (buccal-lingual direction) using a water-cooled diamond saw (ISOMET 1000 Precision Saw, Buehler, Lake Bluff, USA).

The samples were photographed (Microscope Olympus® BX41, Olympus, Tokyo, Japan) at 40× magnification, and images of 5.1 megapixels (Olympus Camedia C-5060, Tokyo, Japan) were obtained. The image analysis software used was Image Pro Plus®, version 4.5 (Media Cybernetics, Silver Spring, USA). Each image was calibrated individually using a standard scale (µm). Three measurements (upper, middle and lower region in the analyzed area) were taken for each image, indicating the depth of the dye infiltration (length of dye penetration), and a mean was calculated for each specimen. The same examiner performed all of the measurements.

Scanning electron microscopy (SEM)

A total of 20 teeth *per* group were evaluated by SEM. All of the specimens were transversely sectioned using a water-cooled diamond saw. The sectioned specimens were washed with 20 mL of distilled water and were ultrasonicated for 10 min. Dehydration was achieved to the critical point of dryness using a graded series of ethanol (25%, 50%, 70%, 90% and 100% for 10 min/each). The specimens were mounted on metal stubs, kept in a 37°C stove for 24 h and stored in a vacuum silica gel desiccator for 48 h. To perform the SEM analysis, the samples were sputter coated with 25 nm of gold for 10 min.

Nine images from each sample (central area) were obtained by SEM (Shimadzu SSX 550®, Shimadzu do Brasil, São Paulo, Brazil), operated at 20 kV with 1,000× and 3,000× magnification. The SEM photomicrographs were evaluated quantita-

tively and qualitatively. The quantitative analysis was performed by counting the number of dentinal tubules and obtaining an estimate *per* mm², considering a total area of 1,600 µm², of the diameter of dentinal tubules and the opened tubular area using Image Pro Plus® software. The qualitative evaluation considered dentin surface characteristics, inter-tubular and peritubular dentin, dentinal tubules and smear layer deposits.

Energy-dispersive X-ray (EDX)

The samples were examined by energy-dispersive X-ray microanalysis (Shimadzu SSX 550®, Shimadzu do Brasil, São Paulo, Brazil) to determine the presence of chemical elements in deposits found next to the dentinal tubules of each specimen. The spectrum was obtained at 20 kV, with a spot size of 5 nm and a counting time of 300 s. This procedure provided qualitative information about the presence of fluorine (F), sodium (Na), magnesium (Mg), silicon (Si), phosphorus (P), potassium (K), strontium (Sr) and calcium (Ca).

Statistical analysis

Intra-examiner reproducibility (dentinal permeability, tubule number, tubule diameter and tubule area) was tested with intraclass correlation coefficients (ICC). Comparisons among the groups regarding dentin permeability and tubule number, diameter and area were performed by one-way ANOVA and Bonferroni's *post hoc* test (GraphPad Prism™ 5.01, San Diego, USA). The significance levels were set at $\alpha = 5\%$ ($p \leq 0.05$).

Results

Reproducibility

The intra-examiner intraclass correlation coefficients (ICC) were 0.98 for dentinal permeability, 0.99 for tubule quantity, 0.97 for tubule diameter and 0.99 for tubule area (good reliability).

Dentin permeability

The means and SDs of dentin permeability are shown in Table 2. There were significant differences among the groups ($p < 0.0001$).

Table 2 - Mean and standard deviation of dentin permeability (5% Evans blue dye) and number and diameter area of dentinal tubules after treatment. DW, control; FT, fluoride toothpaste; SCT, strontium chloride toothpaste; PCT, potassium citrate toothpaste; PNT, potassium nitrate toothpaste.

Groups	Parameters			
	Dentin permeability (μm)	Number of dentinal tubules (1000/ mm^2)	Diameter of dentinal tubules (μm)	Area of dentinal tubules (μm^2)
DW	398.40 \pm 180.90 ^b	73.13 \pm 26.23 ^b	1.26 \pm 0.25 ^b	71.16 \pm 19.83 ^c
FT	302.20 \pm 131.60 ^b	30.43 \pm 7.72 ^a	1.36 \pm 0.25 ^b	26.27 \pm 8.96 ^b
SCT	86.19 \pm 61.19 ^a	23.16 \pm 6.67 ^a	0.93 \pm 0.22 ^a	13.11 \pm 7.31 ^a
PCT	90.02 \pm 55.73 ^a	24.33 \pm 5.80 ^a	0.96 \pm 0.20 ^a	13.69 \pm 6.70 ^a
PNT	69.03 \pm 55.16 ^a	25.92 \pm 7.64 ^a	0.88 \pm 0.24 ^a	14.27 \pm 9.62 ^a

Vertically, different letters denote significant differences ($p < 0.01$) in each parameter (ANOVA and Bonferroni's *post hoc* tests).

Scanning electron microscopy (SEM)

The values of the dentinal tubules *per* mm^2 , the diameter, and the area exhibited significant differences ($p < 0.0001$) among the groups (Table 2).

The qualitative analysis showed the presence of open and partially occluded tubules, peritubular and intertubular deposits, and a few smear layers in the SCT, PCT and PNT groups. In the DW and FT groups, most of the dentinal tubules were opened, with neither deposits nor smear layers on the peritubular and intratubular dentin (Figure 1).

Energy-dispersive X-ray (EDX)

EDX analysis revealed peaks of chemical elements found on both the experimental and control samples (Figure 1).

Discussion

Dentin hypersensitivity in humans can, at present, only be evaluated by extraction, replication or dentin biopsy, all of which have problems and limitations. Animals provide a better option for studying the action of desensitizing agents on dentin. The present study, therefore, used rats because of the ease of obtaining samples and of reproducing oral characteristics comparable to those of humans. The cervical area of the incisors was analyzed because it was more appropriate, due to their greater number of dentinal tubules.¹³⁻¹⁵

The analysis of dentin permeability in this study showed higher dentin permeability in the DW and FT groups. The SCT, PCT and PNT groups had lower dentin permeability. Studies *in vitro* and *in vivo* have shown a reduction of dye penetration af-

ter the application of desensitizing agents.^{3,7} Analysis of dentin permeability using dye diffusion in animal models was deemed appropriate considering the objectives of this study. There is no way to reproduce satisfactorily the pulpar pressure, although other studies have attempted to do so using hydraulic conductivity to analyze dentin permeability.^{4,5,16} While this method does indeed apply constant pressure in an attempt to simulate normal pulpar conditions, it cannot reproduce other crucial factors, such as oral temperature, saliva flow, dental biofilm, and mastication.

This present research showed that the control group had a higher number of opened dentinal tubules; the other experimental groups had lower numbers of opened dentinal tubules, perhaps due to the action of desensitizing and abrasive agents occluding the tubules in the current results. The number of dentinal tubules ranges from 21,000/ mm^2 to 48,000/ mm^2 in human dentin, at a position halfway between the pulp and enamel.^{6,7,15} In this present study, after brushing with different toothpastes, the number of dentin tubules ranged from 23,160/ mm^2 to 30,430/ mm^2 . These results are very similar to those of other studies using desensitizing agents on rat teeth and on human dentin.^{7,17}

The dentin tubule diameter was smallest in teeth that were brushed with desensitizing toothpastes. These results obtained in the SCT, PCT and PNT groups were probably due to the action of desensitizing, in combination with abrasive agents, resulting in higher number of partially obliterated tubules. The rat incisors in the current study showed a dentin tubule diameter ranging from 0.88 to 1.36 μm .

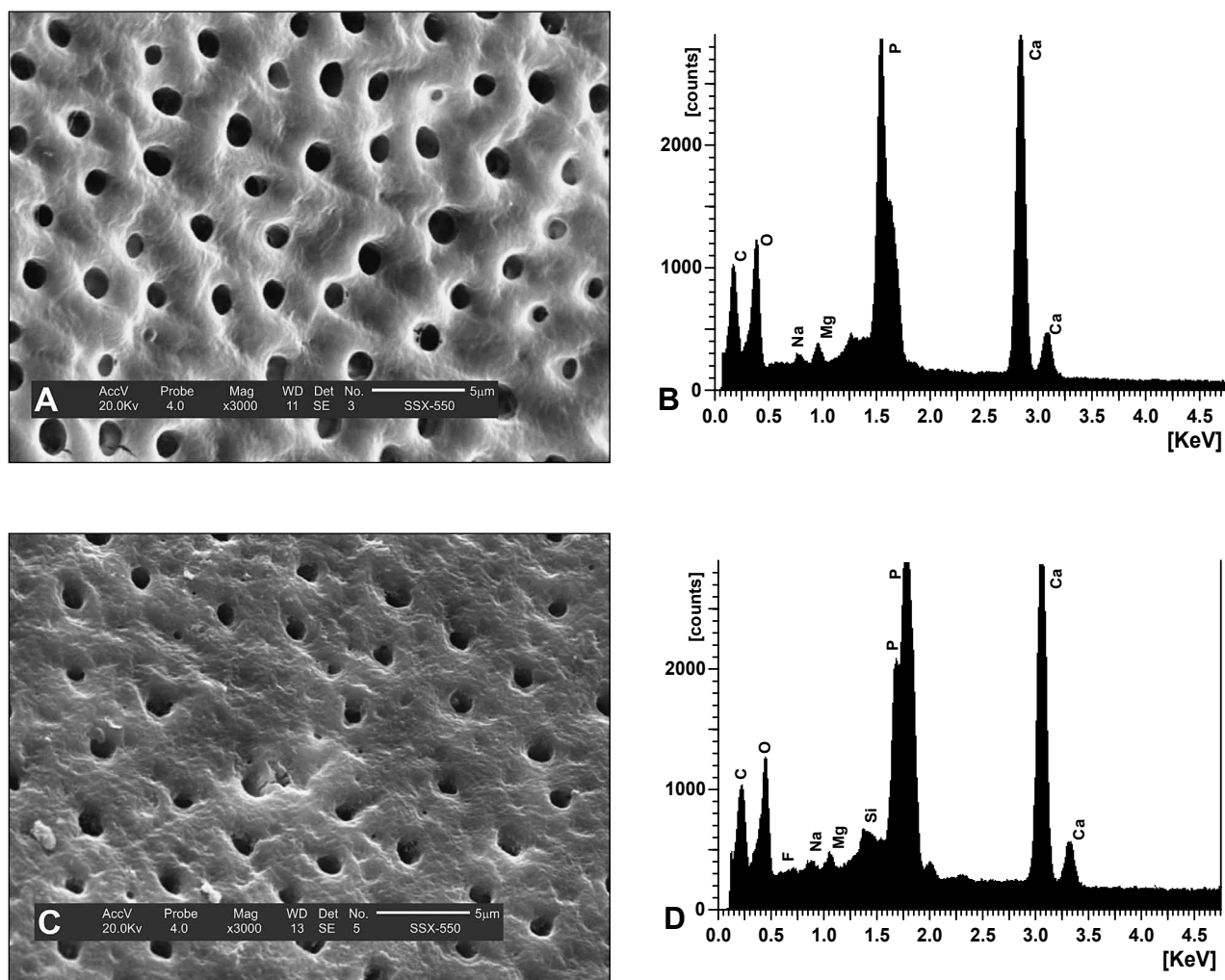


Figure 1 - SEM photomicrographs and the EDX spectrum of the dentin surface after treatments. DW, control: **(A)** Most of the dentinal tubules were opened, with neither deposits nor smear layers on the peritubular and intratubular dentin. **(B)** Presence of P, Ca, Na and Mg. FT, fluoride toothpaste: **(C)** Features similar to those found in DW, with opened dentinal tubules and no deposits or smear layers. **(D)** presence of P, Ca, Na, Si, and Mg and traces of F. [continued on next page]

These values are lower than those in other studies that reported dentin tubule diameter in rats, but in those studies, the dentin remained untreated.^{7,13-15} The differences among studies might be related to the different chemical agents (desensitizing or abrasives) applied to the dentin.

The dentinal tubule area exhibited significant differences among the groups. These findings showed 4.45%, 1.64%, 0.82%, 0.85% and 0.89% of the dentin area occupied by opened tubules in the DW, FT, SCT, PCT and PNT groups, respectively. In humans, it is possible to find 6% of the dentin area occupied by tubules at a distance halfway be-

tween the pulp and enamel.¹⁸ Rat incisors showed 4% to 11% of the corresponding area occupied by tubules.^{14,15} The percentage of the area occupied by dentinal tubules in this study was probably lower due to the use of the toothpastes, which can occlude many tubule orifices. The decreased dentinal tubule diameter in the control group could have been due to the normal presence of minerals in saliva; this factor probably contributes to a reduction in dentin sensitivity.^{4,16}

The samples from the SCT, PCT and PNT groups had dentin tubules that were partially occluded, and there were fewer deposits or smear lay-

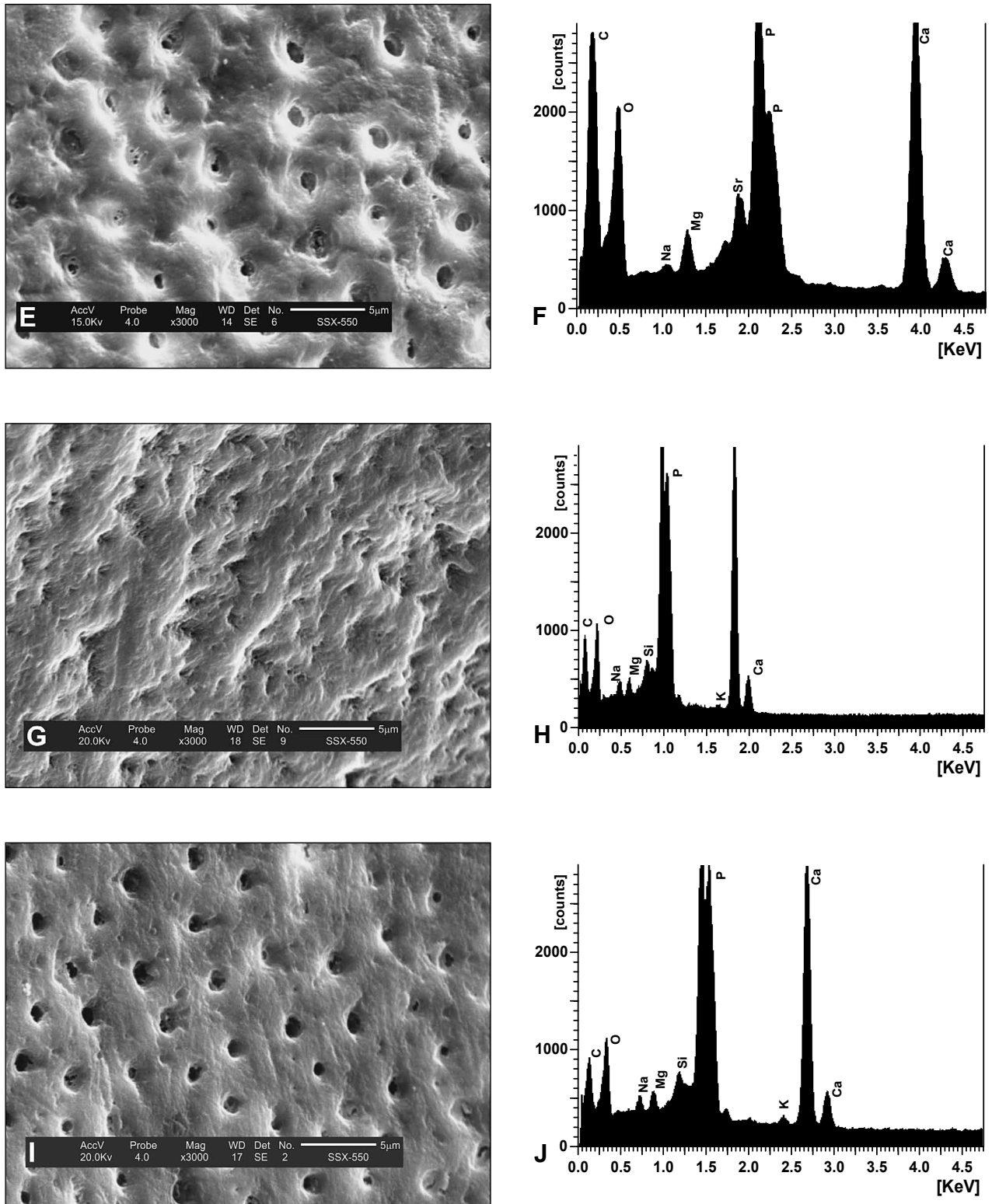


Figure 1 [continued] - SCT, strontium chloride toothpaste: **(E)** Presence of opened and partially occluded tubules, deposits and some smear layers on the dentin. **(F)** Presence of Sr, Na, Ca and Mg. PCT, potassium citrate toothpaste: **(G)** Presence of opened and partially occluded tubules. **(H)** Low quantity of K and presence of Na, Si, Ca and Mg. PNT, potassium nitrate toothpaste: **(I)** Open and partially occluded tubules. **(J)** Traces of K and presence of Na, Si, Ca and Mg.

ers on their peritubular and intertubular dentin. In the DW and FT groups, the majority of dentin tubules were open, and there were no smear layer deposits. Similar results have been observed using fluoridated toothpastes, with no differences between *in vitro* and *in vivo* studies when toothbrushing with water was used as a control.^{1,5}

The dentifrices used in this study were chosen based on their different compositions of desensitizing agents. Potassium citrate and potassium nitrate desensitizing agents are added to toothpastes to interrupt the neural response to painful stimuli.² In contrast, strontium can be absorbed by the enamel and dentin, and it acts by improving fluoride's effects and by occluding dentinal tubules.¹¹ However, the real effects attributable to strontium still remain unclear.²

According to these results, strontium chloride, potassium citrate, and potassium nitrate associated with abrasive agents were able to reduce dentin permeability when compared to brushing with fluoride toothpaste or distilled water. Generally, the retention of abrasive particles is relatively poor; however, silica possesses considerable uptake and occlusion powers and is not easily dislodged by water, let alone by acids.⁸ This retention could be the result of hydroxyl groups' binding of artificial silica to the calcium receptors on the dentin surface; natural silica, being inert, would not display such ionic interactions. Citrates have the ability to chelate calcium. Hence, it is possible that tubular occlusion could occur with these pastes, resulting in the reduction of pain.⁹

The EDX microanalysis of the samples showed only traces of active desensitizing chemical agents

(Sr and P). Si (component of abrasive agent) was present on samples treated with different toothpastes. The presence of calcium, carbon, and oxygen could indicate calcium carbonate (abrasive agent), but these ions are also part of the mineral content of the underlying dentin. These results are in accordance with those of other studies in the literature.^{5,6,9,13} In contrast, another study did not find chemical evidence of the active ingredients of desensitizing agents after EDX analysis.^{5,9} Possible reasons for these different results include the variations in product formulations and concentrations and in application methods.

Several factors could have been responsible for the differences between the results of the current study and other studies in the literature, such as the concentration and formulation of the toothpaste and the time and frequency of brushing, as well as the evaluation methods used.³⁻⁵ Factors such as oral temperature, saliva flow, dental biofilm, and mastication also could have interfered with the action of the desensitizing toothpastes.

Although *in vivo* models are probably more convincing than *in vitro* models, the results of this study should be interpreted with caution before extrapolating them to dental practice in humans, considering the nature and limitations of animal experimentation. Despite the study limitations, the results showed significant differences among desensitizing toothpastes in comparison with other groups

Conclusion

In conclusion, desensitizing toothpastes decreased dentin permeability, although they produced only partial dentin tubule obliteration.

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