Characterization of dentin surface after application of desensitizing agents: SEM analysis

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ABSTRACT

Objective: To analyze the morphology of dentin after application of desensitizing agents. Materials and Methods: Ten third molars were sectioned to obtain 2mm thick dentin disks and divided into 4 groups (n = 5): G₀ – cut with a diamond disk; G₁ – Colgate® Sensitive Pro-relief™ toothpaste; G₂ – Durafur® Fluoride varnish; G₃ – biosilicate aqueous solution (Biosilicato®). After storage in dehydrated silica-gel (37°C/24h), the samples were placed on aluminum supports, coated with gold-palladium and observed under a scanning electron microscope. Post-treatment photomicrographs were obtained of the dentin. Results: In the G₁ group the smear layer was observed; in the G₂ and G₃ groups, both the desensitizing agents and the smear layer and in the G₀ group, a film of varnish. Conclusions: Different morphologies of dentin were observed after the proposed treatments for dentin hypersensitivity.

Keywords: Dental Scaling, Dentin sensitivity, Desensitizing agents, Scanning electron microscopy.

INTRODUCTION

Cervical dentin hypersensitivity is a symptom reported in vitalized teeth with areas of dentin exposed to the oral environment due to degradation of the enamel or cement (1). It is an intense, non-spontaneous, acute, transient, localized, short term pain of rapid onset in response to mechanical, chemical, thermal or osmotic stimuli without involvement of other pathological or structural defects in the teeth (2-4).

It compromises the quality of life (5) and it is the main complaint in the search for access to dental services since it affects 57% of the general population (6). The incidence varies considerably depending on the group being studied and most of them are in the age range 20-40 years, with the peak incidence at the end of the third decade and a decline during the fourth and fifth decades of life (7), due to the decreased permeability of the dentin, the neural sensitivity through sclerosis and the formation of secondary dentin which occurs with advancing age (8). In contrast, with the increase in life expectancy, the prevalence of hypersensitivity may increase as, nowadays, older individuals tend to present more healthier functioning teeth; leading to an increased risk of losing more mineralized tissue in the cervical region and consequently more gingival recession (7).

Gingival recession usually precedes dentin hypersensitivity and is perhaps the most predisposing factor; there is a prevalence of between 60% and 98% in patients with periodontal disease. Scaling and root planing, basic procedures of periodontal therapy, induce the movement of fluid within the dentinal tubule by removing the smear layer. The alteration in intratubular fluid flow activates the mechanical receptors present on the surface of the pulp tissue and this is revealed in patients by the sensation of pain (7).

The control of dentinal hypersensitivity, after removing the coating on the cement, can be accomplished with the application of desensitizing agents.
zation agents to the exposed dentin which have a palliative effect on pain symptoms. The desensitizing agents used for this purpose should; present fast action, ideally with long-term effects; not irritate the pulp; be painless and easy to apply and should not stain the teeth (9). The mode of action is based on blocking the hydrodynamic mechanism by applying substances that obliterate the exposed dentinal tubules or block the neural transmission of painful stimuli (6).

Products which act by obliterating the exposed tubules offer a greater prospect of instant relief from hypersensitivity either by depositing a thin layer of particles or inducing the formation of a natural mineral in situ (6). In the first situation, fine abrasive particles are provided by the dentifrice to form a physical barrier on the exposed dentin surface and open dentinal tubules, such as fluoride-based materials. According to the manufacturers, toothpastes based on Pro-Ar·gin™ technology and Bioglasses, such as biosilicate (VitroVita®), act by blocking neural transmission, promoting the formation of calcium phosphate minerals which adhere to the exposed dentin (6).

The management of dentin hypersensitivity is complex and should take into consideration etiological, preventive and therapeutic aspects (7-9). In order to contribute to the study of this subject, the present study proposed to evaluate the characterization of dentin surfaces after application of known desensitizing agents, testing the hypothesis that similar findings will be find on dentin surfaces.

**Material and Method**

After approval from the Committee for Ethics in Research (protocol No. 0177/10), 10 third molars were disinfected for 7 days in a solution of 0.5% chloramine-T at 4°C. The teeth were cleaned with periodontal curettes and stored in deionized water, refrigerated at 4°C, with weekly changes, until the start of the experiment. With the aid of a diamond disk (Extec 12205, Erios, São Paulo, Brazil) coupled to a cutting machine (ISOMET 1000, Buhler Ltd., Lake Bluff, IL, USA) the occlusal surface was removed (200 rpm refrigerated) and parallel cuts were made in the mesiodistal direction up to the cement-enamel junction to obtain two dentin disks 2 mm thick of the cervical third. This procedure was intended to simulate the production of a *smear layer* on the dentin, similar to that which would occur during periodontal therapy after scaling and root planning (10). The specimens were numbered from 1-20 and allocated into four experimental groups (n = 5), as described in Table 1, for application of the desensitizing agents as described in Table 2.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Desensitizing agent</th>
</tr>
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<tbody>
<tr>
<td>G&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Cut with a diamond disk</td>
</tr>
<tr>
<td>G&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Application of Colgate® Sensitive Pro-relief™ toothpaste.</td>
</tr>
<tr>
<td>G&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Application of Duraflur® Fluoride varnish.</td>
</tr>
<tr>
<td>G&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Application of Biosilicato® aqueous solution.</td>
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</tbody>
</table>

**Table 2 – Materials, composition, action**

<table>
<thead>
<tr>
<th>Material</th>
<th>Composition</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colgate® Sensitive Pro-relief™ (Colgate – Palmolive)</td>
<td>Powder: sodium saccharine, slurry of pumice; Liquid: sodium fluoride, sodium saccharine, ethanol.</td>
<td>Formation of calcium fluoride.</td>
</tr>
<tr>
<td>Duraflur (Dentsply)</td>
<td>8% Arginine, calcium carbonate, fluoride1450 ppm.</td>
<td>Formation of calcium-rich layer.</td>
</tr>
<tr>
<td>Biosilicate (VitroVita)</td>
<td>45% SiO₂; 24.5% Na₂O; 24.5% CaO; 6% P₂O₅</td>
<td>Formation of carbonate hydroxyapatite layer</td>
</tr>
</tbody>
</table>

Throughout the laboratory phase, the dentin disks were stored in *Eppendorf* tubes containing 2 ml of deionized water. At the moment of application of the desensitizing agents, the disks were removed individually, placed on a glass plate and divided into G<sub>1</sub> (cut with a diamond disk); G<sub>2</sub> (rubbed with Colgate Sensitive Pro-relief toothpaste for 15 seconds with a microbrush, left in place for 5 minutes and then washed with 10 ml of distilled water); G<sub>3</sub> (rubbed with Duraflur fluoride varnish for 15 seconds, left in place for 5 minutes and then washed with 10 ml of distilled...
water); G\textsubscript{4} (rubbed with biosilicate aqueous solution in the proportion 0.6g/2mL of distilled water for 15 seconds, left in place for 5 minutes and then washed with 10 ml of distilled water). The excess moisture was removed with absorbent paper and the disks were stored in a desiccator containing silica gel for 24 hours, before being placed on aluminum supports, coated with gold-palladium and taken to the scanning electron microscope (JSMT 220A, Jeol USA, Inc., Peabody, MA, USA) to obtain photomicrographs characterizing the dentin surface after the treatments described.

**Results**

This qualitative evaluation demonstrated different patterns in the surface morphology of the dentin following the treatments, simulating the periodontal scaling procedure. In the G\textsubscript{1} group a smear layer was observed covering the dentin surface (Figure 1). In the G\textsubscript{2} group, after application of the Colgate® Sensitive Pro-relief\textsuperscript{TM} toothpaste, the surface appeared to be covered and the dentinal tubules obliterated (Figure 2), very similar to that observed in the G\textsubscript{1}. In the G\textsubscript{3} group, after application of Duraflur, a seemingly thick coating of material was observed on the dentin surface. The appearance was a little different from the previous images (Figure 3). In the G\textsubscript{4} group, partially obliterated dentinal tubules were observed in the dentin treated with biosilicate aqueous solution in the proportion 0.6g/2 mL of distilled water (Figure 4). No open dentinal tubules were observed, simulating what would happen after scaling and root planing in basic periodontal treatment\textsuperscript{10}.

**Discussion**

The topical application of desensitizing agents on the dentin, as used in this study, has been proposed in order to occlude possibly exposed dentinal tubules (10) and thereby minimize the perception of pain by the patient. Dentin
hypersensitivity deriving from periodontal therapy has variable durability, and procedures for scaling and root planing generate a smear layer on the dentin (11). The results of this study demonstrated different morphologies of the dentin, thus rejecting the hypothesis tested. After scraping, the surface remains covered by a smear layer, but if there is exposure of dentinal tubule tips or very deep regions (Figure 1), the desensitizing agents may act as a physical barrier to the movement of dentinal fluid, and block neural stimulation where applied (11).

Figure 2 shows the dentin treated with Colgate® Sensitive Pro-relief™ toothpaste which was presented to the odontology market as an alternative for the treatment of hypersensitivity. It contains 8% Arginine, Calcium Carbonate, and 1450 ppm of fluoride and acts through exclusive Pro-Argin™ technology by obliterating the open dentinal tubules. According to the manufacturer, the combination of arginine with calcium carbonate creates an alkaline environment, forming a mineral layer rich in calcium on the surface and interior of the dentinal tubules, by precipitation of the calcium and phosphate present in saliva, providing instant relief from the perception of pain with everyday use (6,11).

Previous studies have demonstrated hypersensitivity reduction after a single session (12), with reports of pain reduction, stimulated by touch and application of an air jet, immediately after dental scaling. Twice daily application for 7 days with a cotton-tipped applicator versus a fingertip demonstrated similar reduction in pain and when compared to desensitizing toothpaste containing 2% potassium ion and 1450 ppm fluoride (NaF) demonstrated better results after 2, 4 and 8 weeks (13,14).

Figure 2 depicts a surface covered with a single application of Colgate® Sensitive Pro-relief™ toothpaste for 15 seconds, where the dentin can be observed already covered by the material and the smear layer, which possibly reduces hypersensitivity, and it is expected that subsequent applications of the product would ensure the maintenance of the effect through continuous deposition of the product on the dentin.

Studies have demonstrated the efficacy of fluoride in reducing dentin hypersensitivity (7,8,15). The mode of action of fluoride occurs by precipitation of calcium fluoride crystals within the tubules (8). As the granules of calcium fluoride present larger diameters than the dentinal tubules, the external stimulus cannot reach the pulp to produce the sensation of pain (15), however, these crystals are not resistant to removal through the action of saliva, brushing or food substances (16). If the precipitate is predominantly composed of fluorapatite, it can form stable crystals deposited deeply within the dentinal tubules (17). In this study, the fluoride varnish produced a thick film, distinct from the images observed in the other treatments. The varnish, associated with fluorides has good applicability in the management of dentin hypersensitivity after periodontal therapy, and with a more lasting effect (18). In the form of a varnish, it has low solubility in saliva and higher concentrations of fluoride are slowly released into the oral environment for hours (18) with the advantage of being deposited on the dentin and promoting remineralization. The Duraflur® varnish, represented in figure 3, reinforces these claims as the image differed from those of the other desensitizing agents tested, being the only agent to appear as a uniform and apparently thicker film on the dentin.

This study evaluated a desensitizing agent based on bioglass, Biosilicato® from Vitro Vita®. Its major component is silicate which acts as a nucleus for the precipitation of calcium and phosphate in the formation of hydroxyapatite (17,19), joining chemically with hydroxyapatite. There are reports about its remineralizing potential (6,15), because as it is a bioactive material, it has the ability to bond to living tissues (19). Previous analysis by means of scanning electron microscopy (20) demonstrated that its application on exposed dentinal tubules produced a layer of apatite. Other studies have described its advantage over the other desensitizing agents, as it is the longer lasting effect in reducing pain (15,19).

In the present study, the biosilicate and other desensitizing agents were rubbed for 15 seconds, but different protocols can be followed and influence outcomes. In the case of biosilicate (20), different preparation vehicles (fluoride or water) and application forms (microbrush or Robinson brush) were equally effective in decreasing the sensitivity of the dentin in a clinical study that was applied for a longer period than in our study. When the biosilicate is completely dissolved, the formation of hydroxyapatite apatite (20,21) increases, especially when mixed with distilled water and immersed in artificial saliva for 24 hours.
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(21). Figure 4 of the present study corroborates the results of this research, when observing the dentin surface covered by the product. But its ability to improve bone formation was not observed comparatively to the blood clot (22).

In the present study, the image observed in Figure 2 is similar to the images acquired in Figures 1 and 4, suggesting that periodontal scaling does not fully expose the tubules and the desensitizing action can be effective regardless of the product used.

One of the limitations of this study was the application time of 15 seconds and the waiting time of 5 minutes, which was chosen due to the manufacturer’s recommendations and to standardize the protocols between the products tested. Although the methodology was standardized, additional studies are needed to evaluate whether in the long-term these products remain on the dentin, with different protocols and especially facing acid challenges, since they presented different mechanisms of action. As scaling and root planing do not fully expose the dentinal tubules, it can be considered that the protocol used in this study is sufficient for the treatment of dentin hypersensitivity in this particular situation. Based on the methodology used, it may be concluded that the morphology of dentin among the groups varied according to the desensitizing agent tested.

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Resumo


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