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Original Article

Occlusion of dentinal tubules on periodontally involved teeth by dentifrice containing stannous fluoride and sodium fluoride

Çiğdem Doğan[1)], Hatice S. Yıldırım[1)], Hare Gürsoy[2)], and Leyla Kuru[1)]

[1)] Department of Periodontology, Faculty of Dentistry, Marmara University, Istanbul, Turkey
[2)] Department of Periodontology, Faculty of Dentistry, Yeditepe University, Istanbul, Turkey

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Abstract

Purpose: This study examined the occlusion effect of a dentifrice containing stannous fluoride (SnF_2) and sodium fluoride (NaF) on periodontally involved teeth in comparison with healthy teeth using scanning electron microscopy (SEM) in comparison with a dentifrice containing NaF alone.
Methods: Sixty dentine samples obtained from single-rooted premolars, 15 of them extracted for orthodontic reasons (Group H) and 15 because of periodontal destruction (Group P), were included in the study. Each group of specimens was further divided into subgroups: HC and PC (control), H1 and P1 (treated with SnF_2 and NaF), and H2 and P2 (treated with NaF). The samples were brushed twice a day for 7 days, kept in artificial saliva, and examined by SEM. The diameters of open tubules and the numbers of tubules were assessed at ×2,000 magnification.
Results: The H and P groups showed similar diameters of open tubules. The numbers of open tubules in Groups H1, P1, H2, and P2 were significantly lower than in Groups HC and PC ($P < 0.001$), and consistent with the percentages of occluded tubules. Group P1 had the highest percentage of occluded tubules.
Conclusions: Although both dentifrices were found to successfully occlude dentinal tubules, the dentifrice containing SnF_2 and NaF provided the highest degree of occlusion in periodontally involved teeth.

Keywords: dentifrice, dentine hypersensitivity, occlusion, scanning electron microscopy, stannous fluoride

Introduction

Dentine hypersensitivity is a localized, short, and sharp pain that occurs in response to heat, touch, osmotic or chemical stimuli when the dentine layer is opened to the oral environment as a result of enamel or cementum loss through attrition, abrasion, or erosion; or due to gingival recession [1]. It is well known that patients consulting dental clinics because of periodontitis often suffer from dentine hypersensitivity [2], and that this problem has a tendency to increase following nonsurgical or surgical periodontal therapy for removal of plaque and calculus, and elimination of periodontal pockets [3].

A number of theories have been proposed to explain the underlying mechanism of dentinal hypersensitivity. According to the hydrodynamic theory (the most widely accepted one), fluid movement in exposed dentinal tubules is the mainly responsible for activation of pulpal nerve endings [4]. Dentinal fluid can move outward following thermal changes (especially cold) or dehydration after air stimuli. Similarly, substances such as sugars, acids, and salt can change the osmotic balance, thereby causing fluid movement [5]. Therefore, all treatment options aim at stopping or reducing this movement.

So far, methods used for the treatment of dentinal hypersensitivity have been classified into three main categories: desensitization of nerve endings, anti-inflammatory approaches, and occlusion of dentinal tubules [6], the latter being the most frequently used [7]. Tubule occlusion can be achieved either by the patient at home with desensitizing agents or by a dental professional in the dental office [6]. Patient-applied desensitizing agents include dentifrices containing arginine, strontium, bioactive glass, sodium fluoride (NaF) or stannous fluoride (SnF_2) [8], while in-office treatment methods include application of desensitizing agents, lasers, periodontal tissue grafts and restorations [9].

In 1935, Grossmann [10] suggested some requirements for the ideal desensitizing agent, and these remain valid today. It was suggested that such an agent should not irritate the pulp, be easy to apply, painless when applied, and be consistently effective and quick-acting. Unfortunately, most of the agents currently in use do not fulfil all of these criteria.

Dentifrices containing SnF_2, which has anti-caries and anti-plaque properties, have been shown to be very effective for the treatment of dentinal hypersensitivity [11]. With the activity of saliva, fluoride ions are released from SnF_2 and interact with hydroxyapatite from the dentine structure to form a fluorapatite compound [12]. At the same time, the reaction taking place between SnF_2 and hydroxyapatite results in the formation of $Sn_3PO_4F_3$ and CaF_2 products, which have a high capacity to occlude dentinal tubules [13]. Then, an oxidization process occurs in which the Sn ions change from Sn (II) to Sn (IV) and form insoluble metal oxide plugs to occlude the tubules [12,13].

In vitro studies of extracted periodontally healthy human teeth [14] and bovine teeth [15] have proved that SnF_2 occludes dentinal tubules mechanically. However, as far as is known, no previous study has compared the occluding effect of SnF_2 and NaF in combination on extracted teeth from periodontally healthy subjects and patients with periodontitis. Studies of dentinal tubule occlusion by other desensitizing dentifrices on periodontally involved teeth have also been limited [16]. Therefore, this *in vitro* study was conducted to evaluate the effect of a dentifrice containing a combination of SnF_2 and NaF on dentinal tubules of sound and periodontally involved teeth using a scanning electron microscope (SEM), in comparison with a dentifrice containing NaF only. The null hypothesis tested was that the effects of the dentifrice containing the SnF_2 and NaF combination and the dentifrice containing NaF on dentinal tubule occlusion would not differ between periodontally involved and healthy teeth.

Materials and Methods

Thirty freshly extracted human single-rooted premolars were included in this study with approval from the Ethics Committee of the Faculty of Dentistry, Marmara University (2022/61). Fifteen teeth were obtained from patients with periodontitis, and 15 from periodontally healthy subjects who had undergone tooth extraction for orthodontic reasons. Teeth with cracks, large carious lesions, or restorations were excluded. Prior to preparation of the dentine specimens, the teeth were stored in distilled water with thymol as a preservative to inhibit microbial growth until use. Dentine specimens were prepared in accordance with Cakar et al. [17]. One dentine slice 3 mm thick was prepared from each tooth. Two transverse cuts, the first at the cementoenamel junction and the second 3 mm apical to the first one, were made using a low-speed diamond blade (Isomet; Buehler Ltd., Lake Bluff, IL, USA). The slices were then cut into two halves to obtain a total of 60 specimens, which were placed in 10-mm-deep acrylic resin poured into special molds at a standard height. The bakelites, each containing 6 speci-

Correspondence to Dr. Çiğdem Doğan, Department of Periodontology, Faculty of Dentistry, Marmara University, Recep Tayyip Erdoğan Complex Health Campus, Başıbüyük Street 9/3 34854, Maltepe-Istanbul, Turkey
Fax: +90-216-777-50-01 E-mail: cigdemtaylan@gmail.com

mens, were polished in succession with 600-, 800-, 1,000-, 1,200-, and 2,500-grit silicon carbide polishing papers placed on the polishing machine at 400 rpm until the cementum layer covering the dentinal surfaces had been removed. They were then ultrasonically cleaned using distilled water for 480 s and air-dried. The dentine specimens were treated with 6% citric acid for 90 s to eliminate the smear layer on the dentinal tubule orifices, and then rinsed with distilled water and air-dried.

Thirty dentine specimens obtained from periodontally healthy (H) subjects comprised Group H while 30 dentine specimens obtained from patients with periodontitis (P) comprised Group P. The total of 60 dentine specimens embedded in acrylic resin with exposed dentinal tubule orifices were then further divided into the following 6 subgroups:

Group HC (n = 6): Specimens obtained from periodontally healthy subjects used as untreated controls.

Group PC (n = 6): Specimens obtained from patients with periodontitis used as untreated controls.

Group H1 (n = 12): Specimens obtained from periodontally healthy subjects and treated with dentifrice containing 0.454% SnF_2 and 0.0721% NaF (Sensodyne Sensitivity & Gum; GlaxoSmithKline Consumer Healthcare, Middlesex, UK) (Table 1).

Group P1 (n = 12): Specimens obtained from patients with periodontitis and treated with dentifrice containing 0.454% SnF_2 and 0.0721% NaF.

Group H2 (n = 12): Specimens obtained from periodontally healthy subjects and treated with dentifrice containing 0.15% NaF (Sensodyne Total Care; GlaxoSmithKline Consumer Healthcare, Middlesex, UK) (Table 1).

Group P2 (n = 12): Specimens obtained from patients with periodontitis and treated with dentifrice containing 0.15% NaF.

Table 1 shows the ingredients of the various dentifrices used for dentinal tubule occlusion. Following the application of 1.0 ± 0.1 g dentifrice onto the brush head (FlossAction head, Braun Oral-B Triumph 5000 with a wireless smartguide; Procter & Gamble, Cincinnati, OH, USA), brushing was carried out for 10 s, as described previously [18]. The electric toothbrush was used in daily clean mode with 48,000 pulsations, 10,500 oscillations, and a force not exceeding 2.5 N. The dentifrice was left on the dentine surface for 30 s and then gently washed with distilled water. This procedure was repeated for 7 successive days, and the bakelites containing the dentine discs were then stored in artificial saliva at room temperature. Artificial saliva was freshly prepared according to the composition described by Fusayama et al. [19].

The surface morphology and dentinal tubule occlusion were visualized and analysed using SEM. The dentine specimens were dried for 24 h at room temperature followed by coating with a 55 nm gold/palladium layer in a sputter coater. The gold/palladium-sputtered specimens were placed in the vacuum chamber of the SEM (Evo Ma10; Zeiss Inc., Jena, Germany) and electron micrographs of each specimen were taken at ×2,000 and ×5,000 magnification. The number of dentinal tubules was counted in the centre of each specimen on the photomicrographs [20] using Image J (National Institutes of Health, Bethesda, MD, USA). The diameter of open tubules was measured in AutoCAD (Autodesk Inc., San Francisco, CA, USA).

The SPSS software package (Chicago, IL, USA) was used for statistical analysis. Descriptive statistics such as mean ± standard deviation were calculated for each group. The Kruskal-Wallis test was used for multiple group comparisons, and a post-hoc Mann Whitney U-test with Bonferroni correction was used for comparison of two groups. Statistical significance was set at $P < 0.05$.

Results

The diameters of open tubules, the numbers of open tubules per 100 μm² and the percentages of occluded tubules in all groups are presented in Table 2. Significant inter-group comparisons were detected for all parameters ($P < 0.05$). Almost all dentinal tubule orifices were exposed in both control groups irrespective of whether the teeth had been obtained from healthy subjects or periodontitis patients. These control specimens showed normally structured surfaces without a smear layer (Fig. 1a, b). In contrast to the control groups, all of the treated groups had plugs on the dentinal tubule orifices. Although a small number of tubules in specimens treated with NaF-containing dentifrice remained open (Fig. 1e, f), almost all tubules in specimens treated with dentifrice containing SnF_2 and NaF were occluded (Fig. 1c, d). There was no significant difference between the two control groups (Group HC and PC) in the diameters of open tubules ($P > 0.05$). Although the diameters of open tubules in Groups HC and PC were greater than in Groups H1, P1, and H2, the difference was not significant ($P > 0.05$). The diameters of open tubules narrowed from 1.72 ± 1.17 to 0.57 ± 0.49 in the periodontally healthy groups, and from 1.29 ± 0.51 to 0.59 ± 0.81 in the periodontally involved groups. Only Group P2 presented significantly lower diameters of open tubules than the Group PC ($P < 0.05$). The number of open tubules per 100 μm² in Groups HC and PC was significantly higher than in the other groups ($P < 0.001$). However, the differences between Groups HC and PC, and between Groups H2 and P2, were not significant ($P > 0.05$). Additionally, in all groups, the percentages of occluded tubules were compatible with the numbers of open tubules per 100 μm².

Table 1 Ingredients of dentifrices used in this study

Dentifrice 1	Sensodyne Sensitivity and Gum	stannous fluoride 0.454% w/w, sodium fluoride 0.0721% w/w, glycerin, PEG-8, hydrated silica, pentasodium triphosphate, sodium lauryl sulfate, aroma, titanium dioxide, carbomer, cocamidopropyl betaine, sodium saccharin, limonene. (1,450 ppm fluoride)
Dentifrice 2	Sensodyne Total Care	sodium fluoride 0.15% w/w, potassium nitrate 5%, aqua, hydrated silica, sorbitol, glycerin, pentasodium triphosphate, PEG-6, alumina, aroma, titanium dioxide, sodium methyl cocoyl taurate, cocamidopropyl betaine, xanthan gum, sodium hydroxide, sodium saccharin. (1,450 ppm fluoride)

GlaxoSmithKline Consumer Healthcare, Middlesex, UK

Table 2 Multiple and pairwise comparisons of the diameters of open tubules, number of open tubules and percentage of occluded tubules

	Diameters of open tubules	Number of open tubules per 100 μm²	Percentage of occluded tubules
Group HC mean ± SD median (min-max)	1.72 ± 1.17 1.50 (0.00-3.33)	1.14 ± 0.33 1.14 (0.71-1.68)	0.97 ± 0.55 0.84 (0.56-1.13)
Group PC mean ± SD median (min-max)	1.29 ± 0.51 1.14 (0.80-2.33)	1.33 ± 0.44 1.39 (0.76-1.81)	0.84 ± 0.19 0.84 (0.56-1.13)
Group H1 mean ± SD median (min-max)	0.57 ± 0.49 0.67 (0.00-1.40)	0.02 ± 0.01 0.02 (0.01-0.03)	98.18 ± 0.81 98.12 (96.26-99.42)
Group P1 mean ± SD median (min-max)	0.59 ± 0.81 0.00 (0.00-2.00)	0.00 ± 0.01 0.00 (0.00-0.21)	99.70 ± 0.41 100 (98.91-100)
Group H2 mean ± SD median (min-max)	0.66 ± 1.11 0.00 (0.00-3.33)	0.05 ± 0.02 0.05 (0.02-0.08)	96.07 ± 1.36 96.39 (93.15-97.95)
Group P2 mean ± SD median (min-max)	0.23 ± 0.41 0.00 (0.00-1.30)	0.06 ± 0.03 0.05 (0.01-0.10)	95.64 ± 2.35 96.28 (91.50-98.25)
P^*	0.013	0	0
$P^{\dagger(HC\text{-}PC)}$	0.519	0.485	0.31
$P^{\dagger(H1\text{-}P1)}$	0.648	0	0
$P^{\dagger(H1\text{-}H2)}$	0.338	0	0
$P^{\dagger(H1\text{-}HC)}$	0.046	0	0
$P^{\dagger(P1\text{-}P2)}$	0.271	0	0
$P^{\dagger(P1\text{-}PC)}$	0.066	0	0
$P^{\dagger(H2\text{-}P2)}$	0.518	0.443	0.887
$P^{\dagger(H2\text{-}H1)}$	0.08	0	0
$P^{\dagger(P2\text{-}PC)}$	0.002	0	0

SD, standart deviation; *Kruskal-Wallis test; $P < 0.05$; †Mann-Whitney U-test with Bonferroni correction; $P < 0.05$

Discussion

Dentinal hypersensitivity occurs when dentinal tubules are opened to the oral environment due to exposure of the root surface resulting from gingival recession, periodontal disease or periodontal treatment, as well as enamel loss through abrasion and erosion [1,3]. Patients with periodontal disease frequently complain of dentinal hypersensitivity when visiting a

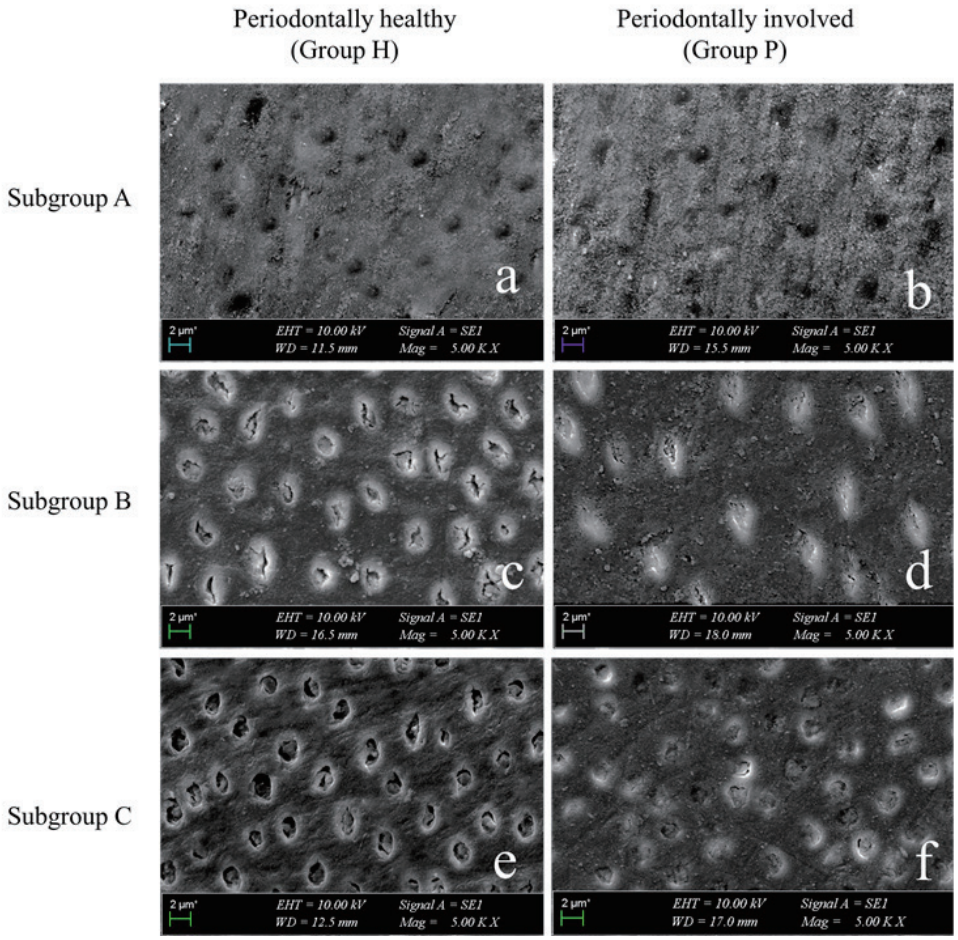

Fig. 1 SEM images of dentine specimens from control specimens (a-b), specimens treated with SnF_2 and NaF (c-d), and specimens treated with NaF (e-f) (×5,000).

dental clinic, and this problem may be exacerbated by periodontal treatment. According to studies of patients suffering from periodontitis, the prevalence of dentinal hypersensitivity is high at 47.8-98% [2,21,22]. Therefore, it has been necessary to assess dentinal tubule occlusion in periodontally involved teeth in comparison with healthy teeth.

The main goal of treatment for dentinal hypersensitivity is complete closure of open dentinal tubules. Patient-applied agents (especially dentifrices) are one of the most accepted and widely used methods for occlusion of dentinal tubules, being non-invasive and easy to handle [8]. It has been reported that several dentifrices containing varying proportions of arginine, bioglass, SnF_2, or strontium chloride have successfully achieved dentinal tubule occlusion [23]. A number of studies have shown that SnF_2-containing dentifrices have an inhibitory effect on plaque formation [24,25]. In addition to *in vitro* studies, clinical studies have also evaluated the effectiveness of SnF_2-containing dentifrices in periodontally healthy subjects. These clinical studies showed that the sensitivity score decreased rapidly and lasted for a long period following the regular use of SnF_2-containing dentifrice [26,27]. NaF is present in desensitizing varnishes at concentrations as high as 2-5% [28-30]. If dentifrices contain a minimal amount of NaF (0.15-0.32%), the tubule occlusion effect is insufficient and it may be improved when combined with different agents such as potassium salts [31]. Consequently, in the present study, it was aimed to evaluate and compare the dentinal tubule occlusion effect of SnF_2 and NaF in combination as a desensitizing agent.

Citric acid is the most frequently preferred decalcification material for elimination of the smear layer [17,20,32-34]. Application of citric acid at concentrations ranging from 1% to 6% achieves decalcification of dentine surfaces. In the present study, application of 6% citric acid for 90 s resulted in removal of the smear layer without damaging the dentine surface, thereby exposing the dentine tubule orifices in both of the control groups (Groups HC and PC). The mean diameter of open tubules was measured as 1.72 ± 1.17 µm and 1.29 ± 0.51 µm in the periodontally healthy control group and the periodontally involved control group, respectively, and the numbers of open tubules per 100 μm^2 were 1.14 ± 0.33 and 1.33 ± 0.44, respectively. The diameters and numbers of open tubules recorded here were lower than in previous studies [17,20,34], which reported that the diameter of open tubules ranged from 2.41 ± 0.11 to 2.57 ± 0.30 µm whereas the number of open tubules per 100 μm^2 ranged from 1.44 ± 0.07 to 1.5 ± 0.23. While previous studies were performed on periodontally healthy third molars, the use of premolars in the present study may have explained the dissimilar results, as different tooth groups may exhibit different tubule patterns [35].

This *in vitro* study found that application of both dentifrices resulted in dentinal tubule occlusion on the orifices of disk specimens obtained from sound teeth. However, the percentage of occluded dentinal tubules in Group H1 (treated with toothpaste containing SnF_2 and NaF), was significantly higher than in Group H2 (treated with toothpaste containing NaF) (98.18 ± 0.81 and 96.07 ± 1.36, respectively), whereas the number of open tubules per 100 μm^2 was correspondingly lower (0.02 ± 0.01 and 0.05 ± 0.02, respectively). The combination of SnF_2 and NaF gave significantly better results. Previous studies have demonstrated that application of a dentifrice containing SnF_2 alone resulted in 50% tubule occlusion [14], whereas the proportion was 83% when SnF_2 and NaF were combined [15]. Thus, the SnF_2 and NaF combination in the present study led to a higher percentage of occluded tubules than in previous studies [14,15,36].

As far as is known, this is the first study to have evaluated the effectiveness of a dentifrice containing both SnF_2 and NaF for occlusion of dentinal tubules in periodontally involved teeth. The percentages of occluded dentinal tubules in Groups P1 and P2 were 99.70 ± 0.41% and 95.64 ± 2.35%, respectively, and the numbers of open tubules per 100 μm^2 were 0.00 ± 0.01 and 0.06 ± 0.03, respectively. These findings demonstrated that both dentifrices successfully occluded tubules in periodontally involved teeth, although the occlusion effect was better for the dentifrice containing SnF_2 and NaF in combination. In addition, the reduction in the diameter of open tubules was markedly higher in periodontally involved teeth than in periodontally healthy ones when this combination was applied, although the difference was not statistically significant.

An early study by Selvig and Zander [37] demonstrated differences in the chemical composition of dentine between periodontally healthy and involved teeth. They reported that dentine in periodontally involved teeth contained higher levels of calcium and magnesium than that in periodontally healthy teeth [37]. In addition, in a later study, Selvig [38] noticed a highly mineralized zone on the clean root surface of periodontally involved teeth. Therefore, it has been suggested that increased mineralization arises through direct contact of root surfaces with minerals in saliva [38]. It is evident that there is an increase in the mineral components of root surfaces when teeth are exposed to the oral environment as a result of periodontal disease.

It has been shown that the reaction between SnF_2-containing agents and hydroxyapatite minerals in dentine tissue gives rise to the formation of several products including $Sn_3PO_4F_3$ and CaF_2 [13,39]. Since periodontally involved and exposed root surfaces show enhanced mineral deposition [37,38], SnF_2 applied to the dentine discs in the present study may have produced high amounts of $Sn_3PO_4F_3$ and CaF_2. This may explain the higher percentage of occluded dentinal tubules in periodontally involved teeth compared to healthy teeth.

The second dentifrice used in the present study also achieved dentinal tubule occlusion despite containing a low concentration of NaF. It is known that NaF can partially occlude dentinal tubules when used together with potassium nitrate [40]. Thus, the combination of potassium nitrate and NaF in this dentifrice may have been responsible for the tubule occlusion in both groups.

The findings demonstrated that both dentifrices achieved marked dentinal tubule occlusion. However, the number of open tubules per 100 μm^2 and the percentage of occluded tubules in the periodontally involved group were significantly higher than in the healthy group. Hence, the null hypothesis that the effect of a dentifrice containing both SnF_2 and NaF and one containing NaF on dentinal tubule occlusion would not differ between periodontally involved and healthy teeth was rejected.

The present study was limited by its *in vitro* nature and therefore did not reflect the perspective of patients regarding the clinical outcome of dentine hypersensitivity treatment. Since physiological and emotional factors may also change the perception of dentine hypersensitivity among individuals, clinical studies are needed to further evaluate the effectiveness of the SnF_2 and NaF combination in periodontitis patients. Other study limitations included the lack of tubule depth measurements and absence of a control for the permanence of occlusion by acid challenge.

Within the limits of this study, both dentifrices occluded dentinal tubules in teeth extracted from healthy subjects and also periodontally involved teeth after an application period of 7 days. Since the dentifrice containing SnF_2 and NaF in combination showed the highest occlusion on periodontally involved teeth, this combination seems promising for daily use in patients with dentinal hypersensitivity due to exposed root surfaces as a result of periodontal disease or periodontal treatment.

Conflict of interest

The authors have no conflicts of interest to declare.

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