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Original Article

Microhardness and Scanning Electron Microscopic Morphology of Permanent Dentin Following the Application of Iranian and Foreign-Made Desensitizing Toothpastes

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Abstract

Background: This study aimed to assess the microhardness and morphology of permanent dentin following the application of Iranian and foreign-made desensitizing toothpastes.

Methods: This in vitro, experimental study evaluated 48 dentin samples prepared of the extracted sound human permanent molars. Dentin samples were randomly divided into three groups (n=16) and subjected to the application of Pooneh Iranian desensitizing toothpaste, Colgate Sensitive Pro-Relief (Poland), and no intervention (negative control). Each dentin sample was then immersed in 5 mL of a demineralizing solution for 10 hours and underwent a demineralization/remineralization cycle (pH cycling) for 14 days. The mean microhardness of the samples was measured at baseline after demineralization and after 14 days. One sample of each toothpaste group was selected for the scanning electron microscopy (SEM) assessment of dentin morphology. Finally, repeated-measures ANOVA was used to analyze the effect of time and type of toothpaste on microhardness using SPSS, version 21.

Results: The comparison of the mean microhardness of the control group with experimental groups revealed no significant difference at baseline or after demineralization ($P>0.05$) although this difference was significant after 14 days ($P<0.001$). The mean microhardness of the two toothpaste groups was not significantly different at different time points ($P>0.05$). Finally, the SEM assessment revealed a greater tubular obstruction in Pooneh group after 14 days.

Conclusions: Pooneh Iranian-made desensitizing toothpaste was comparable to Colgate Sensitive Pro-Relief foreign-made desensitizing toothpaste in terms of the microhardness of permanent dentin. It even demonstrated superior performance with regard to the obstruction of dentinal tubules.

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Highlights

- Iranian-made desensitizing toothpaste was comparable to foreign-made desensitizing toothpaste
- Iranian-made desensitizing toothpaste showed superior performance with regard to the obstruction of dentinal tubules

Background

Dentin hypersensitivity (DH) is a common complaint of patients presenting to dental offices, which more commonly involves the cervical region of the teeth. Maxillary canines and premolars are more typically affected, followed by incisors and molars (1). Its prevalence varies from 15% to 74% in different populations (2) and it is also very common in the European and English populations (3,4). The increased longevity of teeth in the oral cavity (due to improved oral hygiene and dental care services) and the subsequent denuding of root surfaces due to gingival recession or periodontal surgery are among the reasons for the increased prevalence of DH in recent years (5).

DH is characterized by a sharp pain in response to heat, cold, touch, and chemical stimuli, which commonly involves adults who are in the age range of 30-40 years (6-8). The reason is the exposure of dentinal tubules due to enamel loss or gingival recession (9-12). The nutritional regimen and the technique of tooth brushing are among other important etiologies of DH (13,14). DH in the cervical region may occur as the result of mechanical abrasion or occlusal forces (15). The number of exposed dentinal tubules significantly increases (up to 8 times) in patients suffering from DH. In addition, hypersensitized dentin has dentinal tubules with a larger diameter (up to 2 times) compared with normal dentin (16,17).

The hydrodynamic theory is the most widely accepted

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theory regarding the mechanism of DH. According to this theory, the hydrodynamic changes of the intratubular fluid directly stimulate the mechanoreceptors of the pulp or indirectly stimulate the odontoblasts. Thus, any factor decreasing the movement of intratubular fluid or dentin permeability can decrease DH as well (18). In general, different desensitizing treatments decrease the flow of the intratubular fluid or intradental nerve activity by interfering with their conduction velocity (19).

Topical agents such as fluoride, calcium hydroxide, calcium phosphate, silver nitrate, strontium chloride, potassium oxalate, corticosteroids, varnishes, adhesive resins, dentin adhesives, laser, and dentifrices containing potassium nitrate are used for the obstruction of dentinal tubules (20-22).

Toothpastes and mouthwashes are prescribed by dental clinicians for use at home for decreasing DH. Some studies have shown that desensitizing agents such as toothpastes and varnishes cause the obstruction of dentinal tubules and decrease DH (23,24). Promise and Denquel are among the desensitizing toothpastes approved by the American Dental Association, which contain potassium nitrate. Potassium nitrate increases the stimulation threshold of the nerves and prevents nerve depolarization and the influx of sodium ions (25,26). A previous study indicated that the application of toothpastes containing potassium nitrate twice a day caused a significant reduction in DH after 12 weeks (27). Pooneh Iranian desensitizing toothpaste also contains potassium nitrate.

Recently, toothpastes containing arginine were introduced to the market. Arginine plays a role in tubular obstruction and improves DH. Colgate Sensitive Pro-Relief contains arginine (28).

Several techniques have been suggested for the assessment of surface roughness and the other morphological characteristics of dental surfaces, including profilometry, microradiography, scanning electron microscopy (SEM), and atomic force microscopy. SEM is among the most commonly used techniques for this purpose (29).

A high number of foreign-made toothpastes are available in the Iranian market. However, they are costly and cannot be afforded by many patients. On the other hand, information regarding the efficacy of Iranian-made desensitizing toothpastes is limited. Thus, this study sought to assess and compare the microhardness and morphology of permanent dentin following the application of Pooneh Iranian toothpaste (Paksan, Tehran, Iran) and Colgate Sensitive Pro-Relief foreign-made toothpaste (Colgate Palmolive, Poland).

Materials and Methods

This in vitro, experimental study evaluated 48 extracted human sound permanent molars. The teeth with cracks, restorations, caries, abrasion, erosion, or structural defects were excluded from evaluations.

The sample size was calculated to be 16 in each group according to a previous study assuming a 5% level of significance, 80% study power, a mean difference of 14 units, and a standard deviation of 14. The teeth were selected using the convenience sampling method.

The teeth were randomly assigned to three groups (n=16) of control, Colgate toothpaste, and Pooneh toothpaste. The teeth were stored in saline at room temperature until the experiment.

First, the enamel was removed using 008 diamond fissure bur (BTM, Turkey) and high-speed hand-piece under water coolant, and then the surface was polished using 400-2000-grit silicon carbide abrasive papers (Starcke, Matador, Wesserfest, Germany) to expose dentin. Next, the samples were mounted in polystyrene and the microhardness of the samples was measured at three points on the surface under 200 N load for 10 seconds using a Vickers hardness tester (Innova, Netherlands).

Colgate Sensitive Pro-Relief (Colgate Palmolive, Poland), Pooneh Iranian toothpaste (Paksan, Tehran, Iran), and deionized water were used in groups 1, 2, and 3 (as the negative control group), respectively.

Next, two types of solutions were prepared as follows:

1. Demineralizing solution with a pH of 4-4.5 and the following formulation (per each 0.5 L): NaCl (2.9 g), $CaCl_2$ (0.12 g), NaH_2PO_4 (0.13 g), NaF (5 cc; 100 ppm), NaN_3 (5 cc) and acetic acid (1.5 cc);
2. Remineralizing solution (artificial saliva) with a pH of 6.5 to 7 and the following formula (per each 0.5 L): NaCl (2.9 g), $CaCl_2$ (0.12 g), NaH_2PO_4 (0.13 g), NaF (5 cc; 100 ppm), and NaN3 (5 cc).

To create dentin lesions, each sample was immersed in 0.5 mL of demineralizing solution for 10 hours, and the secondary microhardness of the samples was measured by the Vickers hardness tester. After the process of demineralization, the samples were subjected to pH cycling for 14 days (30). In this process, each sample was immersed in the demineralizing solution for 4 hours and then was transferred to the remineralizing solution for 20 hours. Between these two cycles, the samples were exposed to desensitizing toothpaste suspensions (3 parts of artificial saliva and 1 part of toothpaste) twice a day each time for 2 minutes (31). The samples were rinsed with deionizing water for 15 seconds before and after immersion in each solution (31). The teeth were kept in artificial saliva in-between the cycles. After the completion of pH cycling on day 14, the microhardness of the samples was measured again as explained earlier. The samples were incubated at 37°C during this period.

Next, one sample of each group was selected after demineralization and after 14 days, and then gently dried and gold-palladium sputter-coated for SEM assessments. The microphotographs were obtained from the surface at 2X magnification.

Data were analyzed using SPSS, version 21 (SPSS Inc., IL, the USA). Finally, repeated-measures ANOVA was applied to assess the effect of time and type of toothpaste on the microhardness of the samples, and $P<0.05$ was considered statistically significant.

Results

Table 1 presents the mean microhardness of the three groups at the three time points. The results of repeated-measures ANOVA showed that the effect of the type of toothpaste on microhardness was not significant (P=0.95) although the effect of time ($P<0.001$) and the interaction effect of time and type of toothpaste ($P<0.011$) on microhardness were significant.

Table 2 provides the pairwise comparisons of the microhardness in each group at different time points, which revealed significant differences ($P<0.001$). The comparison of the microhardness of the control and experimental groups demonstrated no significant difference at baseline or after demineralization ($P>0.05$) although the difference was significant at 14 days ($P<0.001$) such that the mean microhardness of the control group was significantly lower than that of the toothpaste groups. Based on the comparison of the microhardness of Colgate and Pooneh toothpaste groups, no significant difference was found at any time point (P>0.05). Table 3 summarizes the pairwise comparisons of the microhardness of the three groups at different time points.

SEM assessments revealed that the rate of tubular obstruction after demineralization was the same in Pooneh and Colgate groups. However, tubular obstruction was greater in Pooneh group after 14 days compared with Colgate (Figures 1 and 2).

Table 1. Mean Microhardness of the Three Groups at the Three Time Points

Groups	Baseline (Mean ± SD)	After Demineralization (Mean ± SD)	At 14 Days (Mean ± SD)
Negative control	2.58±48.13	3.07±32.61	1.52±12.36
Colgate	4.73±49.58	3.19±32.73	3.63±27.3
Pooneh	3.36±47.69	2.41±32.58	2.32±29.21

Table 2. Pairwise Comparisons of the Microhardness in Each Group at Different Time Points

Groups	Time Points	Mean Difference	*P* Value
Negative control	Baseline/After demineralization	15.52	<0.001
	Baseline/14 days	35.76	<0.001
	After demineralization/14 days	20.24	<0.001
Colgate	Baseline/After demineralization	16.85	<0.001
	Baseline/14 days	22.28	<0.001
	After demineralization/14 days	5.42	<0.001
Pooneh	Baseline/After demineralization	15.11	<0.001
	Baseline/14 days	18.48	<0.001
	After demineralization/14 days	3.37	<0.001

Table 3. Pairwise Comparisons of the Microhardness of the Three Groups at Different Time Points

Time Point	Groups	Mean Difference	*P* Value
Baseline	Colgate/Pooneh	1.89	0.45
	Pooneh/control	0.4	1.00
	Colgate/control	1.45	0.80
After demineralization	Colgate/Pooneh	0.15	1.00
	Pooneh/control	0.03	1.00
	Colgate/control	0.12	1.00
At 14 days	Colgate/Pooneh	1.90	<0.001
	Pooneh/control	16.84	<0.001
	Colgate/control	14.94	<0.001

Discussion

This study assessed the microhardness and morphology of permanent dentin following the application of Iranian- and foreign-made desensitizing toothpastes. The results showed that Pooneh Iranian-made desensitizing toothpaste was comparable to Colgate Sensitive Pro-Relief foreign-made desensitizing toothpaste in terms of the microhardness of permanent dentin. Both evaluated toothpastes in our study had equal concentrations of sodium monofluorophosphate (1450 ppm fluoride). The present desensitizing agents in the composition of Colgate Sensitive Pro-Relief included arginine and sodium monofluorophosphate while Pooneh toothpaste contained potassium nitrate, zinc citrate, and sodium monofluorophosphate. Potassium nitrate and zinc citrate obstruct the dentinal tubules. Further, Pooneh toothpaste contains silica particles. Tian et al (32) used SEM and demonstrated that nano-silica particles caused an almost complete obstruction of dentinal tubules. These particles have a high potential for DH treatment. However, the available silica particles in the composition of Pooneh toothpaste ranged from 8 to 12 μ in size. Thus, they could not play a role in tubular obstruction.

In our study, SEM results represented that Pooneh toothpaste had superior performance regarding the obstruction of dentinal tubules compared with Colgate arginine-based toothpaste, which is in agreement with the findings of Davies et al, (33), Parkinson and Willson (34), Parkinson et al, (35), and Olley et al (36). They all used SEM and reported that the performance of a strontium acetate toothpaste in the obstruction of dentinal tubules was superior to that of an arginine-based toothpaste. Clinically, arginine absorbs the superficial calcium carbonate to form a positively charged alkaline agglomerate (37). Alkaline agglomerate has high affinity for dentin and deposes salivary calcium and phosphate and the subsequent obstruction of dentinal tubules (37). Alkaline agglomerate and calcium phosphate formed by arginine are easily dissolved following acid exposure (36). Potassium nitrate inhibits the transfer of nerve pulses and obstructs dentinal tubules. Golpasand and Pooneh

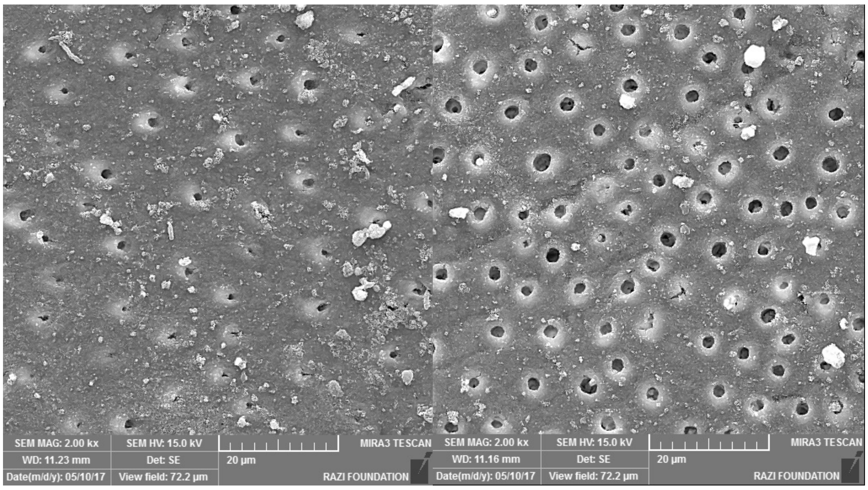

Figure 1. SEM Micrograph of a Sample From Colgate Group After Demineralization (Right) and After 14 Days (Left). *Note*. SEM: Scanning electron microscopy.

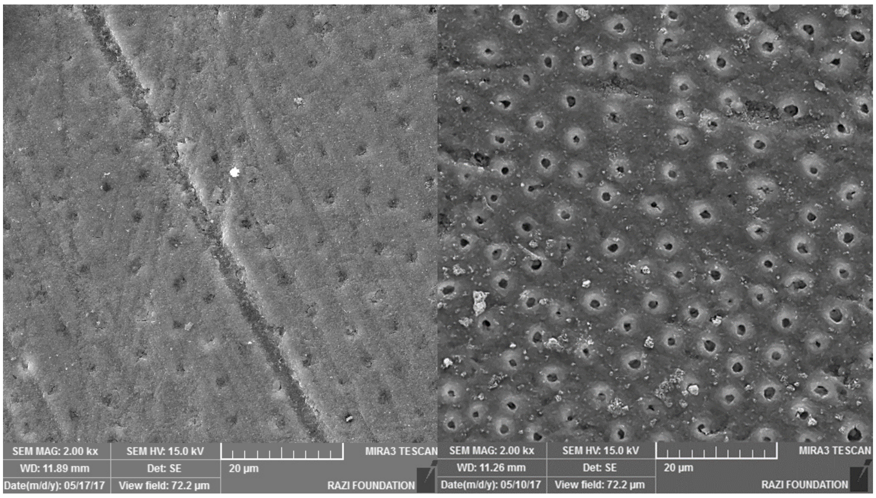

Figure 2. SEM Micrograph of a Sample From Pooneh Group After Demineralization (Right) and After 14 Days (Left). *Note*. SEM: Scanning electron microscopy.

desensitizing toothpastes contain potassium nitrate and are the only Iranian-made desensitizing toothpastes in the market.

Sodium monofluorophosphate creates fluorapatite and decreases the diameter of dentinal tubules and thus resolves DH (38). Citrate anions in the formulation of citric acid, along with sodium citrate and/or polyglycol form a non-ionized complex with calcium cations on the tooth surface. Thus, the calcium citrate complex has been suggested as a tubular obstructing agent (37). Polyglycol decreases DH by depositing salivary proteins and decreasing the diameter of open dentinal tubules. Therefore, toothpastes containing citrate are effective for the treatment of DH (38).

De Oliveira Douglas (39) observed no significant difference in the efficacy of Sensodyne® Rapid-Relief, Colgate® Sensitive Pro-Relief, and Nano P® desensitizing toothpastes after 30 days of use. All treatment groups showed the improvement of DH, and this improvement was smaller in Colgate while greater in Nano P® group immediately after the treatment. Likewise, Wang et al (40) found no significant difference in the efficacy of Colgate Pro-Relief, Desensibilize Nano-P, and Desensibilize Nano-P plus an experimental toothpaste after 1 month and 3 months of use. However, the efficacy of Colgate Pro-Relief significantly increased from 1 month to 3 months. In another study, Cunha et al (41) demonstrated that Colgate Sensitive Pro-Relief caused a lower obstruction of dentinal tubules compared to NovaMin. This can be due to the low concentration of fluoride and the form of the fluoride-containing compound in the composition of Colgate Sensitive Pro-Relief (1440 ppm, sodium monofluorophosphate) compared with NovaMin that contains 12210 ppm fluoride in the form of sodium fluoride. Furthermore, Kulal et al (29) showed that the obstruction of dentinal tubules following the use of nano-hydroxyapatite toothpaste was significantly higher compared to the use of Colgate Sensitive Pro-Relief (containing 1450 ppm fluoride). However, the former had no significant difference with Shy NM toothpaste containing NovaMin. Conversely, the obstruction of dentinal tubules in NovaMin group was higher compared to Colgate Sensitive Pro-Relief. This was due to the higher efficacy of hydroxyapatite for the remineralization of enamel and dentin compared with fluoride. Lopes et al (42) reported that Sensodyne Repair and Protect (1450 ppm fluoride, NovaMin) significantly decreased the number of open dentinal tubules compared with Colgate Total 12 Clean Mint and Colgate Sensitive Pro-Relief. Colgate Sensitive Pro-Relief represented the highest number of open dentinal tubules among all. Based on their findings, the calcium-arginine compound formed after using Colgate Sensitive Pro-Relief had the lowest resistance to washing (42). In their study, Farooq et al (43) concluded that hydroxyapatite toothpaste and Sensodyne Repair and Protect caused a greater obstruction of dentinal tubules compared with Colgate-Palmolive fluoridated toothpaste. They confirmed that hydroxyapatite and bioactive glass have higher remineralizing capability compared to fluoride.

SEM assessments in the current study showed the smaller diameter of open dentinal tubules after 14 days of using Pooneh toothpaste compared with Colgate Sensitive Pro-Relief. Given that the concentration of fluoride is the same in both toothpastes (1450 ppm), a greater tubular obstruction in Pooneh group can be due to the presence of other constituents in the composition of toothpastes. Lopes et al (42) represented that calcium-arginine compounds produced after the application of Colgate Sensitive Pro-Relief had the lowest resistance to washing.

Many previous studies used the microhardness tester for the assessment of surface microhardness (44-46). The Vickers hardness tester was also used in this study to assess dentin microhardness. A direct correlation was found between microhardness and the mineral structure of the tooth (47). According to Angker et al (48), the mechanical properties of dentin depend on its mineral content. Moreover, Arthur et al (49) demonstrated that the percentage of changes in dentin microhardness following the application of Colgate Total (1450 ppm fluoride in the form of sodium fluoride) three times a day was lower compared to its application twice a day. They emphasized that the frequency of using a fluoridated toothpaste is more important in comparison with the amount and concentration of fluoride in its composition. In another study, Diamanti et al (30) reported that a fluoridated toothpaste resulted in higher microhardness compared with a calcium sodium phosphosilicate toothpaste. They also showed that the toothpaste containing 5000 ppm fluoride increased dentin resistance to acid attacks compared with other groups. Higher concentrations of fluoride had higher efficacy in increasing the microhardness. Accordingly, they attributed this finding to a greater crystalized surface coated with fluoride following the use of products with higher concentrations of fluoride because increasing the concentration of fluoride would decrease dentin mineral loss (30).

In the present study, the mean microhardness in both toothpaste groups decreased from baseline to day 14, which is in agreement with the findings of Diamanti et al (31). Additionally, our results revealed no significant difference in the microhardness of the two toothpaste groups at all time points although the mean microhardness of Pooneh group was slightly higher than that of the Colgate group. The lack of a significant difference between the two groups in this respect can be attributed to the equal concentration of fluoride in both toothpastes and similar exposure times (twice a day).

The in vitro design was a limitation of this study that limited the generalization of results to the clinical setting. Thus, future clinical studies are required to assess the efficacy of Pooneh desensitizing toothpaste according to the opinion of the patients.

Conclusions

In general, Pooneh Iranian-made desensitizing toothpaste was comparable to Colgate Sensitive Pro-Relief foreign-made desensitizing toothpaste in terms of the microhardness of permanent dentin. It even showed superior performance with regard to the obstruction of dentinal tubules.

Conflict of Interest Disclosures

The authors declare no conflict of interests.

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Ethical Statement

The study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.RIDS.1394.138).

Authors' Contribution

AV, MJ and HE developed the concept and design of the study. AV and MJ Performed laboratory steps. HE was primarily responsible for writing the manuscript. All authors have read and approved the final.

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