

Assessment of the acid resistance behaviour of dentin tubules occluded by different desensitizers

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Abstract

Aim: To evaluate the efficacy of in-office and home-based products in the prevention of dentinal hypersensitivity on dentin discs.

Methodology: Ninety-six dentin disc samples of 1 mm were divided equally into six groups as follows: Teethmate™, Smartprotect®, novamin®, Arginine calcium carbonate, potassium oxalate with rinse and saline (control). After acid etching, test agents were applied, and eight samples in each group were subjected to citric acid. SEM examination (3000x) and statistical analysis were carried out.

Results: Before citric acid challenge, novamin® and teethmate had superiority when compared to others ($p < 0.001$), but there was no a statistical difference between novamin® and teethmate. After citric acid challenge, teethmate and smartprotect showed resistance on occluded tubules. The decreasing in the level of occluded tubules in novamin® group was statistically significant ($p < 0.05$). In-office products including teethmate and smartprotect were found more successful compared to home-based products. Smartprotect resisted against to citric acid (0.506).

Conclusion: Novamin® showed a successful occlusion in all tubules before acid challenge whereas; it was the most unsuccessful group after acid challenge when compared to other agents. In-office agents were found to be more successful in reducing dentine permeability than home-care products. In-office agents showed more resistance to acid attacks. Further studies are needed to assess the efficacy of desensitizer agents.

Keywords: Dentinal hypersensitivity, in vitro, tubule occlusion, SEM analysis, acid challenge.

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Introduction

The dentin layer is exposed due to the gingival recession caused by an insufficient amount of bone (1), periodontal diseases (2), smoking (3), orthodontic appliances (4), oral piercing (5) and improper toothbrushing (5). As a result of the exposure of the dental tubules to mouth environment, dentinal

hypersensitivity (DH), a short-term and sharp pain which could be triggered by hot, cold and chemical stimuli, may occur (6). Such kind of pain leads to poor quality of life in patients who are influenced in terms of their basic needs such as eating, drinking and maintaining oral hygiene (7,8).

It is known that the hydrodynamic theory is in charge of DH (9). According to this assumption, it is claimed that the fluid movement with the effect of the

above-mentioned stimulants in dentin tubules can increase the pressure over the pulp, thus causing the DH (10). So as to reduce the pain associated with DH, the primary approach is to inhibit the fluid movements by mechanically occluding the tubules (11). In another way, the chemical agents such as potassium nitrate are claimed to block or suppress nerve conduction in dentin tubules (12). Protein precipitation, the laser application, and dentin adhesive agents may be listed as the other methods in the management of DH (13).

The approaches to eliminate DH are divided into two main categories as in-office and home-based approaches. Neither home-based products such as toothpaste and mouth rinse nor in-office agents are not presented as ideal agents. The main task of these agents is to create a barrier in the dentin tubules to prevent temperature transitions and thereby reduce the sensitivity of the dentin. These home-based products include agents with active ingredients such as novamin®, 8% arginine-calcium carbonate (ACC) and potassium oxalate (PO). Novamin®, a commercial name known as calcium sodium phosphosilicate, is effective to occlude dentin permeability (14). Arginine and calcium both in ACC and in saliva constitute a coating in dentin tubules by collaborating calcium and phosphate within the dentin layer (15). Recent studies show that PO can be topically used to occlude dentin tubules (16). These agents can be directly utilized in social life by individuals suffering from dentinal hypersensitivity. Furthermore, the clinicians benefit from professionally applicable certain agents in order to relieve pain in patients with DH. One of these agents containing tetra-calcium phosphate and dicalcium phosphate anhydride is Teethmate® (TM). Another one frequently utilized in dental practice is SmartProtect® (SP), which contains glutaraldehyde, triclosan, olaflur, and 2-propanol providing closure in dentin tubules through its flour content (17).

The objectives of current experimental study were to detect the efficacy of in-office and home-based products in order to constitute the dentin occlusion and to determine the resistance of test products to acid attacks. The null hypothesis of the present study was that there was no difference among the effect of the agents on the dentine discs in terms of occlusion in the dentine tubules and there was no difference in terms of the resistance of the dentinal occlusion created by the agents to the acid challenge.

Materials and Methods

Study design and sample preparation

The ethical approval required for the current study was obtained from the Karabük University non-invasive clinical research ethical committee (4/23, 03/28/2018). This study was carried out on third molar teeth extracted from patients (impacted teeth that are not affected by the external conditions as much as possible) without any restoration and caries. The extracted third molars were conserved in saline until they were examined at the laboratory. Ninety-six dentin disc samples (each disc 1.09 mm \pm 0.16-thick) were obtained from all teeth using the Secotom 50 (Struers, Denmark) cutting device. The occlusal enamel was eliminated from the dentin discs and the surface of disc was kept apart from pulp horns. These samples were divided into six groups with sixteen discs in each group. The first and the second groups were treated with an in-office application such as Teethmate® (group TM) and SmartProtect® (group SP), respectively. The third, fourth and fifth groups were treated with Novamin® (group N), ACC (group ACC), 1.4% PO (group PO) respectively. Lastly, the sixth group was evaluated as control (Group C) and samples in this group were only kept in saline without being treated. All samples were polished using 600 grit silicon carbide paper (Metkon, Turkey) so as to remove intended sections on the disc surface. After obtaining a uniform surface, the dentine discs were preserved in distilled water and sonicated for 20 minutes to eliminate foreign substances. Afterward, the samples were rinsed with saline. In order to open the dentinal tubules, the dentine discs were etched in a Petri dish with 37.5% phosphoric acid (i-dental; Medicinos Linjia UAB, Lithuania) for 30 seconds (Figure 1, 2). After opening the dentinal tubules, the discs were rinsed with distilled water again and sonicated using an ultrasonic cleaner (Alex Machine, Turkey) for ten minutes in order to remove phosphoric acid residue. Before the scanning electron microscope (SEM) analysis, the samples were safely conserved in phosphate-buffered saline (PBS, pH=7) until micrographs are obtained (Fig 3), (Table 1).

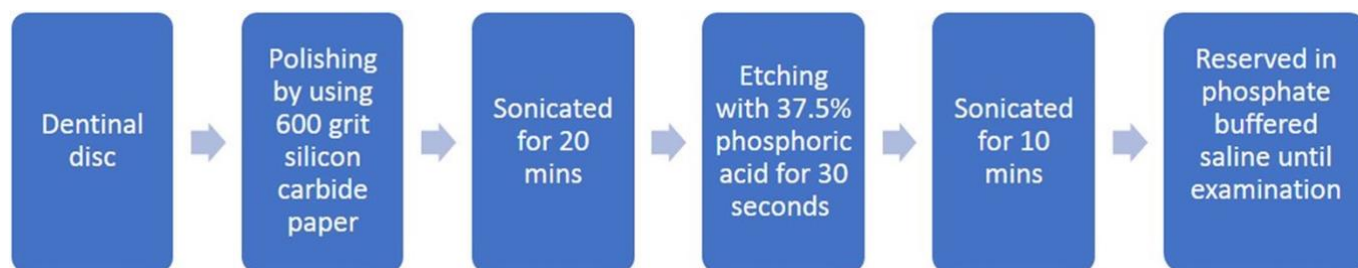


Figure 1. The dentin discs preparation and examination process before SEM analysis

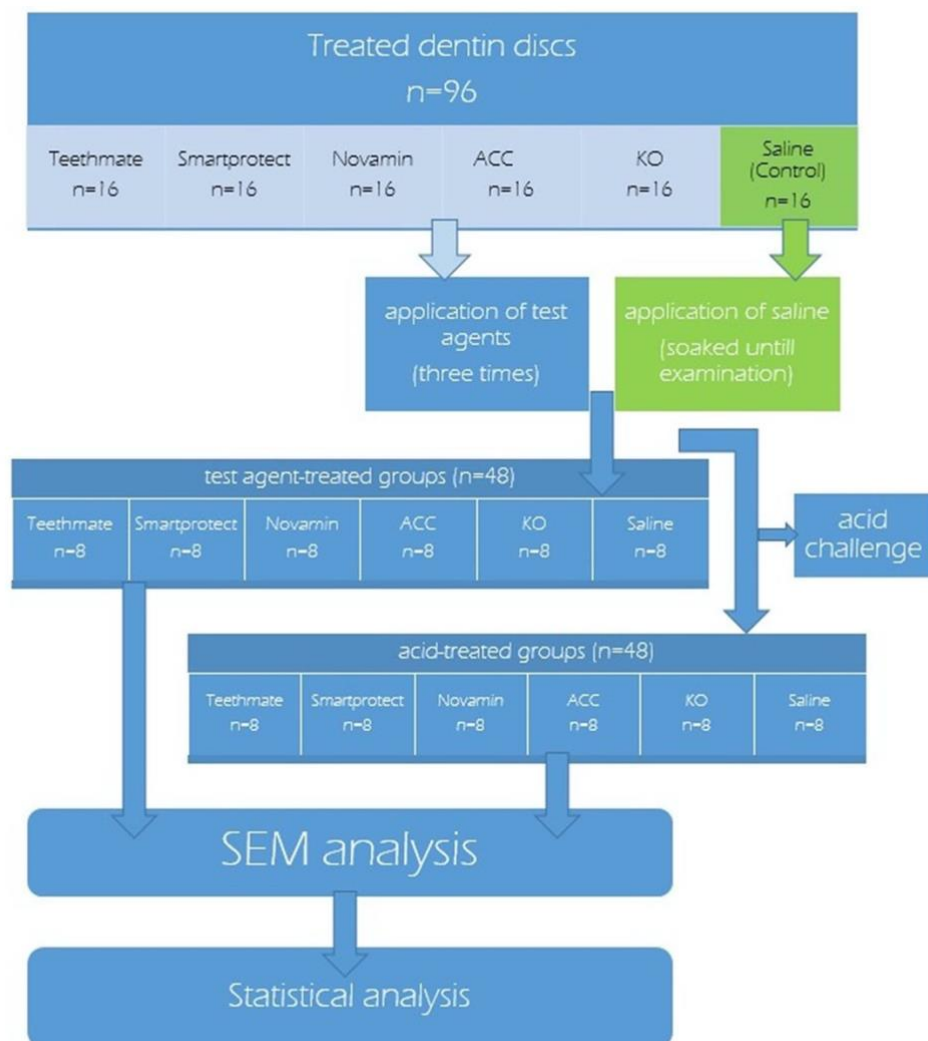


Figure 2. The flow chart of study

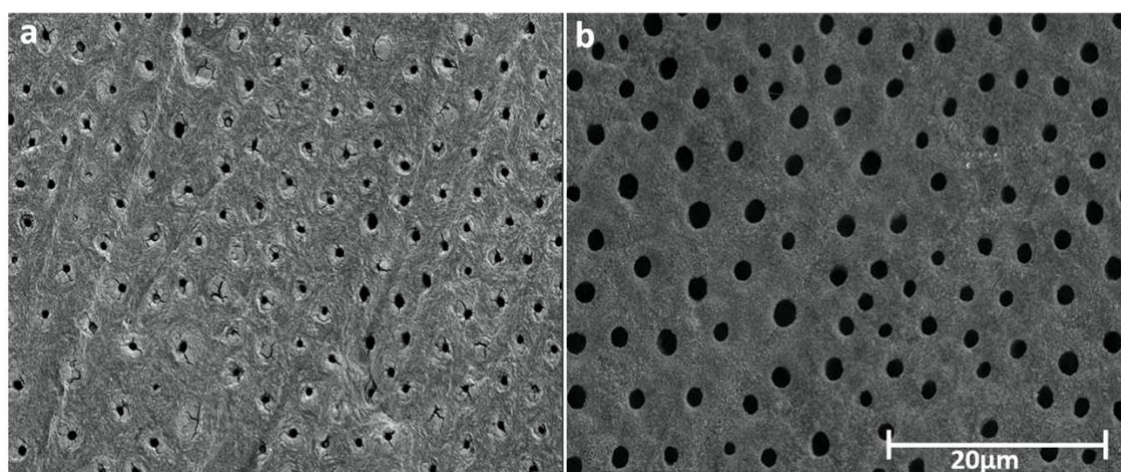


Figure 3. Opening of occluded dentine tubules before applying test agents of dentin discs. a: 37.5% before orthophosphoric acid, b: 37.5% after orthophosphoric acid.

Table 1. Ingredients, manufacturers and usage of the desensitizers used in the study.

Test Agents	Active Ingredients	Producing Company	Usage
Teethmate (In office)	Tetracalcium phosphate, Dicalcium phosphate anhydrous	Teethmate Desensitizer, Kuraray Noritake Dental Inc., Nigata, Japan	Powder and liquid are mixed and for more than 15 seconds and mixture is applied
Smartprotect (In office)	Glutaraldehyde, triclosan, olaflur, 2-propanol in an aqueous solution	Smartprotect®, The Dental Advisor, Detax, Ettlingen, Germany	Dry dentin disc surface is applied with a smart brush for 20 seconds.
Novamin (Home-based)	Calcium sodium phosphosilicate (NOVAMIN)	Sensodyne®, SmithKline Beecham Inc., Berks, England	Gently applied to dentine surfaces with a soft toothbrush
ACC (Home-based)	Arginine (8%), Calcium carbonate,	Colgate® Sensitive Pro- Relief™, Colgate Oral Pharmaceuticals Inc., New York, NY, USA	Gently applied to dentine surfaces with a soft toothbrush
Potassium oxalate (Home-based)	1.4% Dipotassium oxalate	Listerine®, Johnson & Johnson, Sezanne, France	The dentin discs are shaken in 10 ml of liquid for 60 seconds.

Application of test agents

In the experimental procedure, all the samples were treated with PBS, and both TM and SP were applied onto smooth dentine surface according to the manufacturer's recommendation. Novamin® and ACC were kindly applied onto the surface of the dentin discs. The samples in group PO were subjected to the PO-containing rinse in a Petri dish according to manufacturer's instructions. After the applications, the samples were kept at room temperature for five minutes. After this, the samples were transferred to PBS for five minutes. Finally; the samples were rinsed with distilled water. This process was repeated three times (18). Half of the sixteen samples in each group were randomly selected in order to be evaluated for SEM analysis. The selected samples were prepared for SEM analysis through being coated with a layer of gold with a sputter coater (Quorum, United Kingdom).

Acid challenge

The remaining samples from each group were placed in 6% citric acid (pH=2.1) in a Petri dish for one minute and then rinsed in saline for two minutes. Finally, the acid-exposed samples were coated with a layer of gold for SEM analysis (Fig. 4).

SEM analysis

SEM analysis was carried out using the Zeiss Ultraplus SEM machine (Zeiss, Germany). SEM images were taken at 3000x magnification. 10 kV electron beam voltage was used for coated samples and 2 kV electron beam voltage for uncoated samples. Charge

compensation equipment was also used for uncoated samples. During the SEM analysis, the micrographs were taken from the dentin discs for certain circumstances. Firstly, they were taken following acid etching to confirm whether the dentinal tubules were fully opened. After the micrographs were taken from the uncoated samples, test agents were applied. Secondly, SEM analysis was carried out to examine the effectiveness of the test groups. Thirdly, micrographs were taken from the dentin discs to evaluate the effect of the citric acid challenge on occlusion in dentinal tubules. For each one of the examined dentin discs, six non-adjacent images were taken. The assessment was carried out by scoring. According to this scoring: score 1 was given for the case of 100% occluded tubules, score 2 for mostly occluded (50- <100% occluded), score 3 for partial occluded (25- <50% occluded), score 4 for little occluded (<25% occluded), and lastly score 5 for non-occluded (0% occluded).

Statistical Analysis

Data were analysed through IBM SPSS v23. Compliance with normal distribution was examined with Shapiro Wilk test. The intra-class correlation coefficient (ICC) was used for the similarity evaluation between the reviewers. Since the similarity between the reviewers was high, comparisons were made by averaging according to groups. Kruskal-Wallis test was used in the comparison of the data with abnormal distribution per groups. Wilcoxon test was used in the comparison of intragroup results before and after citric acid challenge.

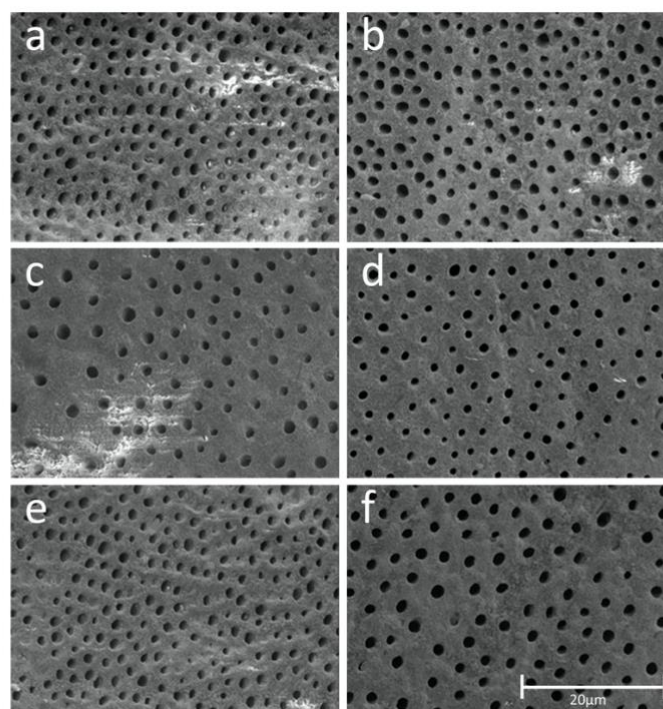


Figure 4. The etching with 37.5% orthophosphoric acid of samples by groups before the application of test agents. a: TM, b: SP, c: Novamin, d: ACC, e: PO, f: Control

Results

Before achieving the statistical results of the scores obtained in this study; the consistency between the scores recorded by the examiners was evaluated. It was observed that there was a strong positive relationship between the examiners. The consistency was found as respectively 0.952 and 0.910 before and after citric acid challenge (Table 2). In the statistical analysis, median values for all the test agents were obtained, and it was investigated whether there was a statistical significance on a median value. Between

professionally applied test agents, group TM was found to be more effective than group SP in terms of the tubule occlusion ($p=0.000$). However, it was observed that group SP occlusion was more resistant to citric acid attack than that of group TM and the difference between these agents was statistically significant ($p<0.001$). In the comparison of the groups regarding the resistance against acid attack; the change in group TM was statistically significant while there was no change in the resistance of group SP ($p<0.001$, $p=0.506$). (Table 3 and 5).

Table 2. Concordance between examiners

	Acid exposure			
	Before expose to citric acid		After expose to citric acid	
	ICC (%95 CI)	P	ICC (%95 CI)	p
Total	0.952 (0.939-0.962)	<0.001	0.910 (0.886-0.928)	<0.001

ICC: Intraclass correlation coefficient, statistical significance level <0.05

Table 3. Comparison of inter- and intra-groups median values after application of agent and acid

Study groups	after agent	after acid	p
Teethmate	1.3 (1 - 3) ^a	2,5 (1 - 4,5) ^a	*0,001
Smartprotect	3 (1 - 5) ^{bc}	3 (2 - 5) ^{ab}	0,506
Novamin	1 (1 - 2,5) ^a	4 (2 - 5) ^{bc}	*0,001
Arginine calcium carbonate	2,5 (1 - 5) ^b	3,5 (2 - 5) ^{ab}	*0,001
Potassium oxalate	3,5 (2,5 - 5) ^c	3,5 (2 - 5) ^{ab}	*0,001
Control	5 (5 - 5) ^d	5 (5 - 5) ^d	1,000

Different letters show the difference between groups, values in parenthesis present lower and upper limits, significance level; <0,05

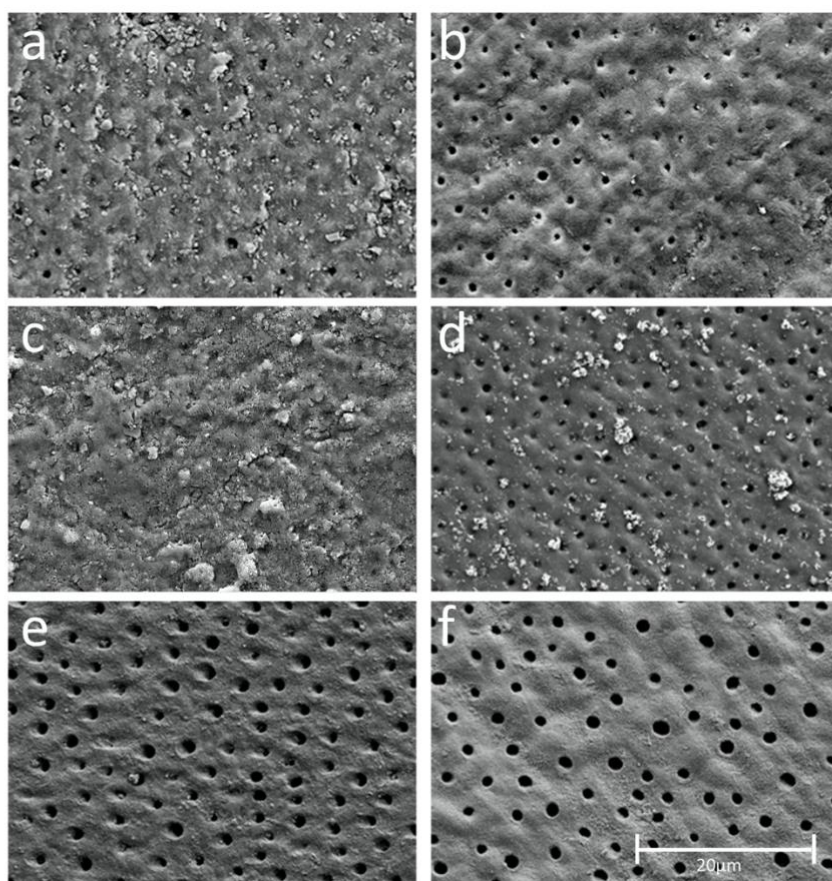


Fig 5. The view of samples after the application of test agents. While the dentin tubules were completely occluded by test agents in Fig 5a and 5c, the image in 5b and 5d showed nearly half-occluded dentinal tubules. The test agent in fig 5e showed no efficacy on dentinal tubules for occluding like control group. a: TM, b: SP, c: Novamin, d: ACC, e: PO, f: Control

For the home-based products, the percentage of occlusion in dentin tubules was highest in group N while group ACC and group PO showed a lower level of occlusion. Before the citric acid treatment, there were statistically significant differences among these three products ($p=0.000$, $p=0.000$, $p=0.013$). After citric acid application, group N showed a dramatic decrease in median value, and its dentinal occlusion could not resist against acid attack. However, while group ACC and PO lost some resistance to the acid attack this decrease was not much in the group N. In-group comparison of resistance against acid attack demonstrated that the alterations in these three products were statistically significant ($p=0.45$).

Before the citric acid challenge, when in-office and home-based products were evaluated together, the highest percentage of dentinal occlusion was found to be group N, TM, ACC, SP and PO, respectively. According to these results, the difference between group N and TM were statistically insignificant, but they showed significant results compared to other test agents ($p<0.05$). In the same way, group ACC and SP did not show statistically significant results. However; while group ACC showed a statistically powerful difference compared to group N, TM, and PO ($p<0.05$); group SP showed a statistically significant difference compared to group N and TM ($p=0.000$), but it showed

no difference compared to group PO. Group PO showed no statistical difference only when compared to group SP (Fig. 5) (Table 5).

After the citric acid challenge, there were also no statistically significant differences between some test agents. For instance, there was no statistically significant difference among group TM, SP and ACC. On the other hand, group TM showed a statistically powerful difference compared to group N and PO ($p=0.011$, $p=0.000$). The quantitative differences between group SP, ACC and N were not statistically significant, and group SP and ACC showed a significant difference compared to group PO ($p=0.000$, $p=0.045$) (Fig. 6). Finally, differences in group N and PO were statistically insignificant (Fig. 7 and 8).

As to resistance of the test agents against citric acid, group SP continued its effect on occluded tubules and showed resistance against acid. Group PO and ACC have shown resistance although not as much as group SP. However, there was a statistically significant difference among these three groups ($p<0.001$). The differences in group N and SP with group N and TM were statistically significant ($p<0.001$). On the other hand, the differences between group ACC and TM were not statistically significant. In addition, group TM and N were statistically insignificant ($p<0.001$) (Table 4, 5).

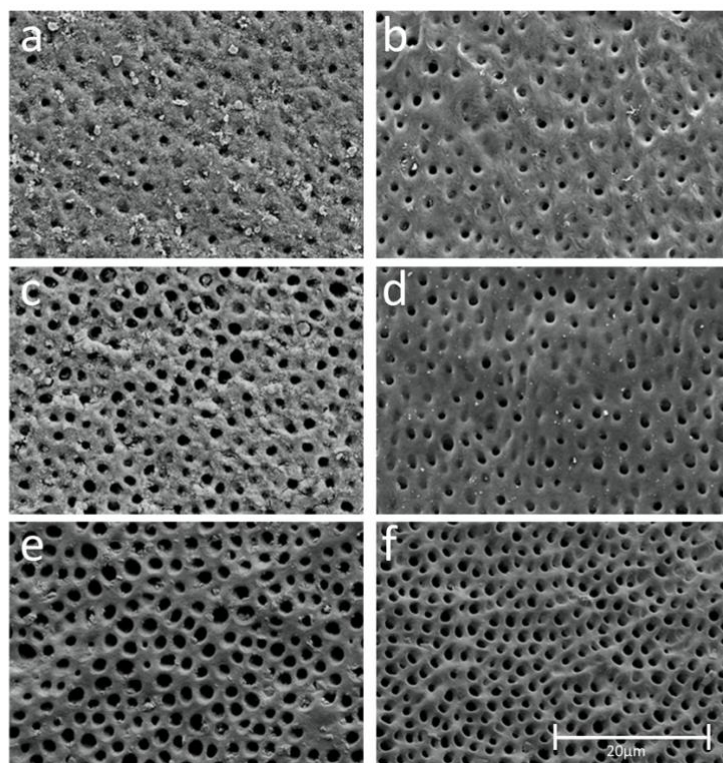


Figure 6. The view of samples after exposure of citric acid. The test agent in fig 5b sustained efficacy on dentinal tubules but especially fig 5c showed failure to preserve the occluding of the dentinal tubules. a: TM, b: SP, c: Novamin, d: ACC, e: PO, f: Control

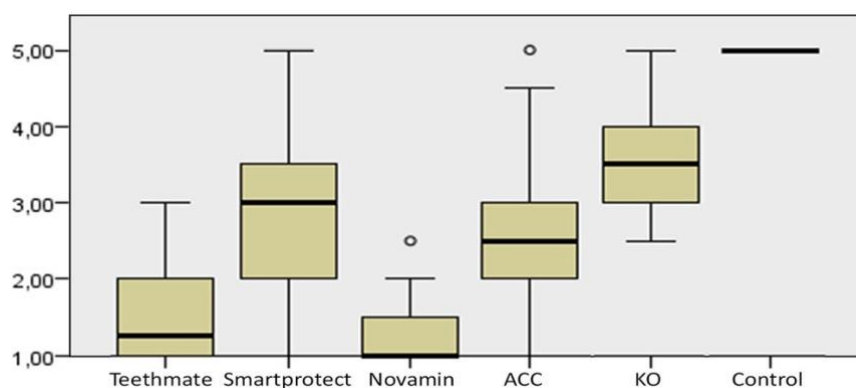


Figure 7. The graphical projection of scoring on samples in respect of groups before application of test agents

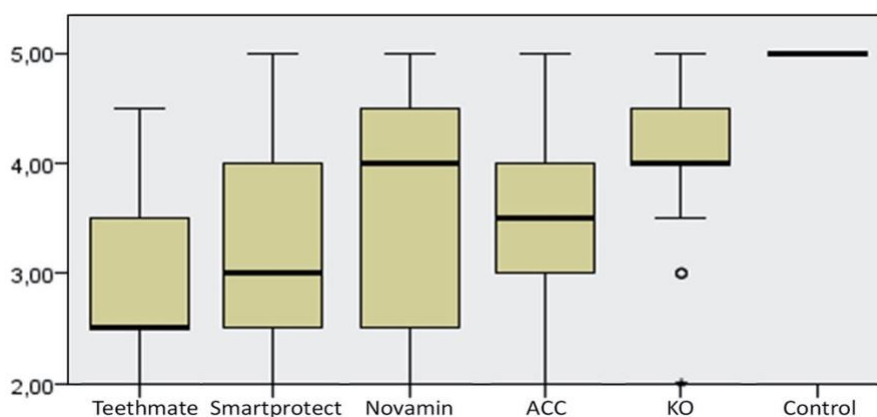


Figure 8. The graphical projection of scoring on samples in respect of groups after application of test agents.

Table 4. Comparison of alterations occurred in tubules after acid exposure with respect to tubule occlusion after application of agents.

Study groups	The level of change
Teethmate	1 (-1 - 3) ^{ac}
Smartprotect	0 (-3 - 4) ^b
Novamin	2,3 (0 - 4) ^a
Arginine calcium carbonate	0,5 (-2 - 4) ^{bc}
Potassium oxalate	0,5 (-2 - 3) ^b
Control (saline)	0 (0 - 0) ^b

Same superscripts show indifference among groups, statistical significance level; <0,05

Table 5. p values of desensitizers comparisons

Groups	Before acid/ After acid (p values)					
	Teethmate	Smartprotect	Novamin	ACC	Potassium oxalate	Control
Teethmate	-	.000*/1.000	1.000/.011*	.006*/.091	.000*/.000*	.000*/.000*
Smartprotect	.000*/1.000	-	.000*/.545	1.000/1.000	.799/.000*	.000*/.000*
Novamin	1.000/.011*	.000*/.545	-	.000*/1.000	.000*/.304	.000*/.000*
ACC	.006*/.091	1.000/1.000	.000*/.545	-	.013*/.045*	.000*/.000*
Potassium oxalate	.000*/.000*	.799/.000*	.000*/.304	.013*/.045*	-	.000*/.000*
Control	.000*/.000*	.000*/.000*	.000*/.000*	.000*/.000*	.000*/.000*	-

Discussion

DH is an important problem in dentistry and has a prevalence of 4-74% (19). Toothpaste, mouth rinses, and several dental products act by releasing antimicrobial and anti-sensitive agents on dentin tubules. Saliva, which acts as a lavation and protective in the mouth, performs the blockage of the dentin tubules with calcium and phosphate ions. However, in the DH occurring causing by reasons such as tooth abrasion and tooth erosion, the calcium and phosphate ions in saliva are inadequate because of slow-release, and so there is a need for an external intake to prevent the dentinal sensitivity (18). In this study, the efficacy of five different desensitizer materials was evaluated.

According to the present study, superiority in group N and TM materials were similar to other studies before the citric acid challenge. In some in vitro studies; researchers have suggested that novamin® toothpaste was more efficient on dentinal tubules (20-24) than Pro-Argin™, which have also been demonstrated in clinical studies (25, 26). However, there are some studies stating that novamin® was not superior to ACC and even failed to close the dentin tubules (18,27,28). The superiority of TM compared to ACC which has been suggested in this study is supported by other studies (29,30).

Novamin® containing dentifrice shows the effect with the sodium and calcium phosphosilicate compound on dentin tubules. This mechanism creates an affinitive effect for collagen and thus occludes the dentinal tubules (31,32). Upon TM application on dentin tubules, calcium and phosphate ions reach saturation and spontaneously form hydroxyapatite (33,34). Some studies showed that crystallized products penetrated

into the dentin tubules (30,35).After citric acid challenge, TM test agent protected the gains in the occlusion of dentin tubules. According to current studies, it could be claimed that TM influenced the dentin tubules with the process of crystallization. This process is explained as follow: the mixed compound of tetra calcium phosphate and dicalcium phosphate anhydrous in the TM material is applied to the surface of dry dentin, and then the resulting compound is converted into hydroxyapatite in aqueous environment (36,37).

Smartprotect group was the second test agent providing the blockage of the dentin tubules after the acid attack following TM but there was no statistical difference between these agents. SP has not lost its effectiveness even after acid challenge. SP contained the fluoride occludes the dentin tubules through a certain mechanism, by which triggering the accumulation of CaF₂, thus on dentin layer decreases the permeability of dentinal tubules (38). In addition, the fluoride has been shown to form a strong barrier against citric acid (39). TM agent has been shown to be resistant against acid applications in other studies, and the results were similar to this study (30,33). In this study, not only the toothpaste with Novamin® containing dentifrice has dramatically lost its effectiveness on the dentinal tubules after citric acid challenge, but also ACC toothpaste lost its efficiency. Although ACC agent showed superiority compared to novamin®, there was no statistical difference between these agents. On the other hands, there was statistically significant differences in the dentinal occlusion results of Novamin®, ACC and PO agents when compared to that of the control group (saline). These results were accordance to other studies. For

instance, an in vitro study performed by Chen et al. (18), demonstrated that the efficacy in dentine tubules against acid attack between Novamin® and ACC were not statistically significant but superior to saline. Lavenden et al. (40) and Li et al. (41) claimed that the occlusion of ACC was more resistant to acid challenge than that of Novamin®. In another in situ study, West et al. (23) evaluated the efficiency of ACC and Novamin® samples in the oral environment after obtaining dentin discs. The dentinal occlusion constituted by ACC was more persistent than that of Novamin®. However, as in the current study, the difference was not significant. In another in vitro study, Bakri et al. (22) stated that Novamin® and ACC were statistically superior to saline. Unlike the results of this study, they reported that Novamin® was more resistant to acid attack than ACC. Parkinson et al. (42) also asserted that Novamin® toothpaste was superior to ACC toothpaste. The different results obtained in this study may be caused by the different duration of acid challenge applied in this study, and also by the methodological differences. The results obtained from some clinical studies presented the similarly with results of this in vitro study, in which not observing any difference between novamin® and ACC agents (28,43).

The first in vitro study published in 2013 regarding PO agent showed favourable results when compared to desensitizing toothpaste and PO-containing mouth rinse was found considerably successful in terms of resistance against acid (44). However, in the clinical study performed by the same researcher, it was stated that PO was exceeded by Sensodyne original in terms of patients' comfort (45). In this in vitro study, PO was relatively less successful compared to other desensitizing test agents for the occlusion of dentin tubules.

The limitation of this experimental study is that the results obtained in dentin discs may be controversial due to the elimination of the factors in clinical conditions. The findings in clinical trials to monitor DH indicate that after improvements in oral hygiene, calcium, phosphate and proteins in saliva can lead to more accumulation with increased contact between saliva and dentin surface, thereby increasing the occlusion of dentin tubules (46). According to the mechanism of ACC; toothpastes containing a combination of calcium carbonate and arginine reduce the occurring DH in the presence of calcium, phosphate and salivary glycoproteins (47). Arginine, a naturally occurring amino acid in saliva, acts simultaneously with calcium carbonate and phosphate to form a plug-in in the dentinal tubules, thereby preventing fluid flow (48). Since this study is not a clinical trial, the potential effect of ACC may not have been observed. The reason for the differences in the results obtained in this experimental study from the clinical results in other studies is related to these factors.

Conclusions

It was evaluated the different desensitizing agents as home-care and professional-care in this study. Thus, TM presented the most successful results after agent

application. However, SP was the most resistant test agent against acid attacks. Although the Novamin® had the successful occlusion agent in dentine tubules, it lost almost whole efficiency after acid attacks.

Ethical Approval: Ethics committee approval was received for this study from Karabük University, Ethics Committee in accordance the World Medical Association Declaration of Helsinki, with the approval number: 2018/4-23.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception - F.K.; Design - FK., O.F.A.; Supervision - F.K.; Materials - F.K., O.F.A.; Data Collection and/or Processing - F.K., O.F.A.; Analysis and/or Interpretation - O.F.A.; Literature Review - FK., O.F.A.; Writer - FK., O.F.A.; Critical Review - O.F.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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