Evaluation of Dentinal Changes Following Application of Three Different Desensitizing Agents

SIMONA STOLERIU¹, GALINA PANCU¹*, ANGELA GHIORGHE¹, DORINA CERASELLA SINCAR², SORINA SOLOMON¹, SORIN ANDRIAN¹, GIANINA IOVAN¹

¹Grigore T. Popa University of Medicine and Pharmacy Iaºi, Faculty of Dental Medicine, 16 Universitatii Str., 700115, Iasi, Romania ²Dunarea de Jos University of Galati, 47 Domneasca Str., 800008, Galati, Romania

The aim of this in vitro study was to evaluate the effect of three desensitizing tooth pastes on the dentinal tubule occlusion. Thirty dentin discs having a thickness of 3 mm were obtained by cutting human teeth. The discs were submersed in citric acid for 30 seconds to open the dentin tubules. Then the discs were cut in two halves. In each group 10 halves were kept in 0.9% NaCl solution) and the other 10 halves were exposed to the action of one of the tested desensitizing toothpastes. The dentin samples were placed in the machine designed to simulate tooth brushing. Three commercial desensitizing toothpastes were chosen to be applied on dentin surface. The morphology of dentin samples and the level of tubule occlusion was scored using scanning electron microscope. All the three desensitizing toothpastes demonstrated significant effects on dentinal tubule occlusion. The tooth paste containing arginine and calcium carbonate as active ingredients showed the highest degree of tubule occlusion, followed by the dentifrice containing strontium acetate and sodium fluoride.

Keywords: dentin desensitizing agents, tubule occlusion, arginine, strontium acetate, stannous fluoride

According to the most widely accepted definition, dentin hypersensitivity (DH) represents a short, sharp pain which results as a response of exposing dentin to various stimuli (thermal, tactile, osmotic or chemical) and cannot be ascribed to any other form of dental defect or pathology [1]. Previous studies have reported a large variation of the prevalence of DH, up to 57% of the adults [2] and 10-20% of the general population being affected by this pathology [3]. The exposure of cervical dentin is a common condition for the onset of DH. Dental trauma, gingival recession, periodontal treatments, aggressive tooth brushing can lead to dentin exposure. Frequent exposure to acidic beverages might determine erosions with high demineralization of exposed dentin and enlargement of the dentinal tubules [4].

There are many studies that support the efficiency of the remineralizing products in the reduction of dental hard tissues demineralization [5-7].

However these commercially available products are not always effective in releasing dental hypersensitivity, especially on short term. Therefore new products have been developed to address this disturbing pathology by mechanisms specifically designed to counteract the DH phenomena.

Many theories have been developed during time to explain the DH. According to hydrodynamic theory [8], an external stimulus applied on dentin surface determines the activation of mechanoreceptors at the pulp/dentin interface due to the rapid fluid flow in the dentinal tubules, which leads to the pain. This hypothesis is sustained by the observation that number and diameter of dentin tubules seem to correlate to DH. It was demonstrated that in hypersensitive dentin the number of tubules is higher and the tubules are larger when compared to sound dentin [9].

Two therapeutic strategies have been used to reduce or to eliminate DH: blocking neural transmission at the pulpal tissues by chemically depolarizing the nerve synapse and occlusion of the dentin tubules [10]. The first mechanism is still controversial, because of the long diffusion distance and outward flow of the dentinal fluid. On the other hand the occlusion of dentinal tubules is well documented and might be obtained physiologically, by mineral crystals formation in the intratubular area due to dentinal fluids and saliva [11] or therapeutically by application of chemical agents. The occlusion of dentin tubules influences the hypersensitivity in two different ways: by blocking the tubules which decrease the dentinal fluid flow and by creating a barrier against the stimuli from the oral cavity.

A large variety of active ingredients were included in the at-home or professional products used for dentin desensitizing: fluorides, strontium salts, arginine and calcium carbonate, oxalates, calcium phosphosilicates, nanoparticles like bioreactive glass. Various mechanisms are involved with controversial results. Strontium salts are absorbed by dentin and form strontium apatite [12], while arginine combined with calcium carbonate can occlude the dentin tubules with calcium phosphate [13, 14] and stannous fluoride produces an acid-resistant precipitate on dentin surface [10]. Phosphosilicates precipitate onto dentin collagen and create deposits located on the dentin surface and in the dentin tubules [15,16] and oxalates form calcium crystals within the dentin tubules and might block the dentinal fluid flow [17]. Fluoride which is the most common component of toothpastes might increase the mineralization of hydroxyapatite [18].

Numerous studies have evaluated various products and therapeutical protocols for desensitizing teeth. Still there is no agreement about the most effective products and application method. A recent systematic review concluded that there is no agent that can provide complete relief from dentinal hypersensitivity [19]. Considering these controversial results, the aims of our study were to analyze the morphological changes of dentin and to assess the degree of tubules occlusion when 3 commercial desensitizing toothpastes having different active ingredients were used.

Experimental part

Samples preparation

Fifty caries-free human teeth extracted for orthodontic reasons were used for this study. The teeth were obtained

from the Department of Maxillofacial Surgery of the Faculty of Dental Medicine. All the teeth were stored in 0.9% NaCl until the start of the experiment. Thirty dentin discs having a thickness of 3 mm were obtained by cutting the teeth in the mesial-distal direction perpendicular to the long axis of the teeth, using diamond discs (Komet Dental, Brasseler GmbH&Co, Germany) at low speed under cooling water. The discs were randomly and equally assigned to three groups (groups 1-3). All the discs were submersed in citric acid for 30 s to open the dentin tubules. Then the discs were cut in two halves. In each group 10 halves were kept in 0.9% NaCl solution (control groups 1-3) and the other 10 halves were exposed to the action of one of the tested desensitizing toothpastes (study groups 1-3). The dentin samples were placed in the machine designed to simulate the forward and backward movements during tooth brushing with amplitude of 30 mm (15 mm in each direction) and frequency of 60 cycles/min.

Toothbrushes having medium hardness of the bristles were used to simulate the regular tooth brushing (Colgate Classic Deep Clean). The load applied on each toothbrush was 250 g. The calculated mean number of toothbrush cycles during a tooth brushing of 2 minutes was 20. In order to simulated the toothbrush for 2 min, twice a day, 30 days, all the samples in the study groups were brushed continuously for 2 h, which is in accordance with the protocols used in previous studies [20].

Three commercial desensitizing toothpastes were chosen to be applied on dentin surface: Sensodyne Repair and Protect (Glaxo Smith Kline) (Group 1), Sensodyne Rapid Release (Glaxo Smith Kline) (Group 2) and Colgate Sensitive Pro Relief (Colgate) (Group 3). The active and inactive ingredients of the toothpastes are summarized in table 1. Slurries were prepared by mixing water and toothpaste (1:2 by volume) and dispensed on the samples surface with a frequency of 1mL\min.

SEM evaluation of the samples and assessment of dentin tubules occlusion

All the prepared samples in control and study groups were evaluated using scanning electron microscope VEGA II LSH (Tescan Czech Republic). Ten standardized images of each dentin surface were obtained at a magnification of 1000X. Two different examiners, trained before the experiment and blinded to the materials used, evaluated the morphology of dentin samples and scored the level of tubule occlusion on a scale from 1–5 according to the tubule occlusion classification scoring system (West, Davies): 1-occluded (100% of tubules occluded); 2-mostly occluded (50–<100% of tubules occluded); 3-partially occluded (25–<50% of tubules occluded); 4-mostly unoccluded (<25% of tubules occluded); 5-unoccluded (0%, no tubule occlusion). The two examiners confronted the score given for each image and the final score resulted as a common decision of both examiners. For each sample the mean score of ten assessment was used for analysis.

Results and discussions

SEM micrographs of dentin samples in all control groups showed large opening of dentin tubules (fig. 1.a-c).

In the Study Group 1, some of the dentin tubules seemed to be totally or partially occluded, whilst some remained opened (fig.1d). Evaluation of dentin surface in Study Group 2 showed the dentin surface covered by particle deposition and very few opened tubules (fig.1e). The samples in the Study Group 3 showed most of the dentin tubules occluded and large deposition of mineral particles on the intertubular dentin (fig.1f). The particle deposition was obvious in Study Groups 2 and 3 when compared to Study Group 1.

The mean scores of tubule occlusion for the control and study groups are presented in table 2. In all the study groups the score values were lower when compared to control, as a result of increased tubule occlusion. Group 3 presented the lowest values of the score and Group 1 the highest.

The values distribution in groups was evaluated using Shapiro-Wilk normality test. A *p* value lower than the chosen alpha level of 0.05 rejected the null hypothesis of normal distribution of values in group. Due to the fact that in Study Group 1 the variable was not normally distributed (table 3), non-parametrical Mann-Whitney U test was chosen to compare the results in the groups.

Toothpaste Active		Inactive ingredients	Producer	Batch no]
Sensodyne	Stannous fluoride	glycerin, PEG-8, hydrated silica,	Glaxo		1
Repair and	0.454% (0.15%	pentasodium triphosphate, sodium	Smith		
protect	w/w fluoride ion)	lauryl sulfate, flavor, titanium dioxide,	Kline		
		polyacrylic acid, cocamidopropyl			
		betaine, sodium saccharin			Table 1
Sensodyne	Strontium acetate	Aqua, Sorbitol, Hydrated Silica,	Glaxo		THE
Rapid	hemihydrate	Glycerin, Sodium Methyl Cocoyl	Smith		INGREDIENTS
	8.0% w/w,	Taurate, Xanthan Gum, Titanium	Kline		OF THE
	sodium fluoride	Dioxide, Aroma, Sodium Saccharin,			TOOTHPASTES
	0.23% w/w	Sodium Propylparaben, Sodium			USED IN THE
	(0.104% w/w	Methylparaben, Limonene			
	fluoride 10n).		-		
Colgate	Arginine 8%	Calcium Carbonate, Aqua, Sorbitol,	Colgate		
Sensitive Pro		Bicarbonate, Sodium Lauryl Sulfate,			
Relief		Sodium Monofluorophosphate (1450			
		ppm F), Aroma, Cellulose Gum,			
		Sodium Bicarbonate, Tetrasodium			
		Pyrophosphate, Titanium Dioxide,			
		Benzyl Alcohol, Sodium Saccharin,			
		Xanthan Gum, Limonene.			J

	Group 1	Group 3	Group 3	Table 2
Control	5.00	5.00	5.00	MEAN SCORES OF TUBULE OCCLUSION FOR CONTROL
Study	3,25	2.67	2.12	AND STUDI GROUPS



Fig.1. SEM micrographs of dentin samples in control groups 1-3 (a-c) and study groups 1-3 (d-e)

Tests of Normality^b

	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	Df	Sig.
group1	.268	10	.040	.829	10	.033
group2	.224	10	.168	.911	10	.287
group3	.240	10	.108	.858	10	.073

a. Lilliefors Significance Correction

b. control is constant. It has been omitted.

	z	Asymp. Sig. (2-tailed)
Control-Group 1 study	-4.058	0.000
Control-Group 2 study	-4.065	0.000
Control-Group 3 study	-4.065	0.000
Group 1-Group 2 study	-3.817	0.000
Group 2-Group 3 study	-3.823	0.000
Group 1-Group 3 study	-3.817	0.000

Significantly statistical differences were obtained when compared the tubule occlusion scores in study groups and control groups (table 4). Also, significantly statistical differences were obtained when compared the values

between the study groups (table 4). The in-vitro assessment of dentinal blockage by dentin disc model has been widely used for evaluating the efficiency of hypersensitivity treatment [21].

Absi et al. reported that in sensitive teeth the number of tubules per unit area is approximately 8 times higher and the diameter of tubules is approximately two times increased than in nonsensitive dentin [22]. In order to simulate these morphological features of hypersensitive dentin we applied citric acid for 30 s on the surface of each dentin sample which resulted in complete opening of the dentin tubules. In order to eliminate variables related to the number and diameter of tubules in different teeth and different areas, we used the two halves of the same dentin specimen for creating the control and the study groups.

Our results suggested that all the tested products determined a significant occlusion of the dentinal tubules.

 Table 4

 RESULTS OF MANN-WHITNEY U

 STATISTICAL TEST

 Table 3

 RESULTS OF NORMALITY SHAPIRO-WILK TEST

The ascending sequence of the mean scores of tubule occlusion was: Colgate Sensitive Pro-Relief; Sensodyne Rapid; Sensodyne Repair and Protec,t with statistical significant differences among all groups. Our results were consistent with several previous studies and inconsistent with others, each of them testing at least one of the dentifrices or one of their active ingredient.

Arnold et all compared the number of dentin tubules after using 6 toothpastes including Sensodyne Repair, Sensodyne Rapid and Elmex Sensitive Professional which had similar active ingredients as Colgate Sensitive Pro-Relief (Pro-Argin and calcium carbonate) [20]. Significant differences of the tubule occlusion after brushing with the toothpaste comparing with the specimens brushed with artificial saliva were found only for Sensodyne Rapid. When comparing to the positive control represented by specimens brushed with conventional toothpaste, the differences were significant for both Sensodyne Repair and Sensodyne Rapid but not for Elmex Sensitive Professional. The different technique of evaluation might explain the controversial results. The SEM examination combined with EDS analysis demonstrated irregular coverage of dentin surface by silica, which is an abrasive used in dentifrice and not an active ingredient. A scattered thin layer of silicon covered the dentin surface in case of Elmex Sensodyne Professional but not in the case of Sensodyne Repair. In our study, an irregular smear layer was observed in all the study groups, the deposition being more signifficant in case of Sensodyne Rapid and Colgate Sensitive Pro-Relief, however the composition of these remnants was not analyzed in our study.

However the better results achieved with Colgate Sensitive Pro-Relief in our study are supported by other researchers. Most of the studies demonstrated a good occluding effect of Pro-Argin products on open dentin tubules [16, 23, 24] which also explain the results of the clinical studies showing an instant relief of dentin hypersensitivity [25]. The two active ingredients, arginine and calcium, are found naturally in saliva and might have a role in natural occlusion of the dentinal tubules and formation of the protective layer on the dentin surface [2]. Chen et al found that applying Colgate Sensitive Pro-Relief on dentin samples resulted in significantly more tubule occlusion when compared to Novamin and salin [26]. The mean score of tubule occlusion was 2.45, which is close to our result. They concluded that even after short term application the tubules of the samples were occluded by crystal-like deposition.

Our results are also in agreement with previous in vitro studies [13, 14], demonstrating that arginine combined with calcium carbonate occlude dentine tubules. Swapna Mahale et al found that after 7 and 14 days of applying Arginine, clear tubule occlusion was noticed [27]. This was thought to be caused by deposition of the calcium carbonate and arginine agglomeration within the tubules. These findings explain the results of clinical trials showing the efficacy of arginine - calcium carbonate in relieving DH [28, 29]. The mechanism of action was explained by Kleinberg who suggested that the combination of arginine and calcium carbonate forms a positively charged complex which readily binds to the negative charged dentin surface and within the dentin tubules. In addition the alkaline pH of arginine-calcium carbonate facilitates the deposition of calcium and phosphate from saliva/and or dentinal fluid to form plugs that seal the patent tubules [30]. In vitro studies suggested that the deposit converts to calcium phospahate [13,14]. The remineralisation process consists in the precipitation of calcium and phosphate as insoluble Calcium Phosphate which, when present in the saliva, is brought to the demineralised enamel in incipient defects resulted from surface demineralization [31].

As regarding Sensodyne Rapid, strontium occurs naturally within human enamel and dentin as a trace element, is a remineralizing agent, and gets incorporated into the mineral phase of enamel by replacing calcium within the apatitic lattice. The occlusion of the tubules can be explained by the affinity for dentin and possibility for adsorption into or onto the organic tissues [27]. In vitro studies have shown that strontium acetate treatments forms small crystalline deposition on the dentin surface [32].

The data about the efficiency of stannous fluoride, which is the main active ingredient of Sensodyne Repair and Protect are controversial [33, 34]. In Arnold's study, no dentinal occlusion of the cross-sections of dentin could be observed after the treatment, however the examination of the surface revealed occluded tubules which is in accordance with our results [20]. In our study all the tested dentifrices had a significant effect on dentin in terms of occluded tubules. Whether the blockage of the tubules at the dentin surface is a result of the active ingredients or also a consequence of the retention of the abrasive agents of toothpastes or smear layer formation is still a matter of debate. Both active ingredients and passive mechanisms may contribute to the desensitizing effect on short term. However, the persistance of the desensitizing effect in the oral environment on long term is probably dependent on several characteristics such as resistance to corrosion, abrasion and and formation of occluding tags into the tubules. Further experiments to investigate these characteristics are necessary for the assessment of long-term effects of theses desensitizing toothpastes.

Conclusions

All the three desensitizing toothpastes demonstrated significant effects on dentinal tubule occlusion. The tooth paste containing arginine and calcium carbonate as active ingredients showed the highest degree of tubule occlusion, followed by the dentifrice containing strontium acetate and sodium fluoride.

Further research is required to analyze the influence of the application protocol on the results and to evaluate the durability of the effects under chemical and mechanical challenges.

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