Comparative Evaluation of Strontium Chloride Desensitizer and Novel Proarginin Desensitizer on Dentinal Tubule Occlusion-A Scanning Electron Microscopic Study

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Abstract: Background: Dentinal hypersensitivity is the commonest dental problem. For this,the products that occlude the dentinal tubules are most widely accepted. Objective: The objective of the present study was to compare the effects of a well-established desensitizing dentifrice containing 2% strontium chloride with a new product 8% arginine on the dentinal tubules, following repeated brushing strokes on a dentine disc model. Methods: For the study, freshly extracted premolars were sectioned at the level of CEJ. The prepared dentin discs were cleaned in an ultrasonic cleaner, polished and etched with 0.5 M EDTA for 2 minutes to expose dentin tubule orifices. The samples were randomly divided into 3 groups: Group 1: control, Group 2: 2% strontium chloride and Group 3:8% pro-argin. The treatment consisted of application with the test dentifrices to the dentin discs followed by preparation for scanning electron microscopic examination. Results: The numbers of dentinal tubules open, partially occluded and completely occluded were counted and statistical analysis was obtained using Post-Hoc ANOVA test. Group 3 i.e. 8% arginine and calcium carbonate group showed highest complete tubule, with a statistically significant difference from group 1 and group 2. Conclusion: Proargin technology appears more promising in providing immediate relief as well as could be more effective on further application than strontium chloride based formulation. [Mayur P NJIRM 2016; 7(6): 85-92]

Key words: Dentinal Tubules, Dentifrices, Hypersensitivity, occlusion

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Introduction: Tooth hypersensitivity caused by exposed dentinal tubules is common in the adult population with as many as one in seven patients presenting for dental treatment [1]. Essentially, exposure of the dentin results from one of two processes: either removal of the enamel covering the crown of the tooth or denudation of the root surface by loss of cementum and overlying periodontium.

Several theories have been cited to explain the mechanism involved in dentinal hypersensitivity. The transducer theory, the modulation theory, the "gate" control and vibration theory, and the hydrodynamic theory have all been presented and discussed throughout the years. The latter, "hydrodynamic theory", developed in the 1960's and based upon two decades of research, is widely accepted as the cause of tooth sensitivity [2]. Assumptions of the hydrodynamic theory conclude that when the fluids within the dentinal tubules absent of a smear layer are subjected to thermal, chemical, tactile or evaporative stimuli, the movement stimulates a nerve receptor sensitive to pressure, which leads to the transmission of the stimuli and ultimate pain response [3]. Alterations in the temperature, pressure, solute concentrations and local charges may result in increased pain, most probably by effecting the direction or rate of flow of dentinal fluid. Therefore if the principles of hydrodynamics are acknowledged, the two most appropriate methods for treating sensitivity are either to physically block the tubules or to desensitize the nerves.

There are two primary approaches to treating sensitive teeth. One is to interfere with nerve transmission, and potassium salts are commonly used to achieve this end. It is believed that potassium depolarizes the nerve fibers and this, subsequently, interferes with the transmission of the pain response [4-6]Most over-the-counter toothpastes for relief of dentin hypersensitivity are formulated with a potassium salt. To be effective, the potassium ions must diffuse through the dentinal tubules against a positive flow of dentin fluid, and must build up and be maintained at elevated concentration in order for the nerve fibers to remain in a depolarized state. This build-up takes time; typically, it is necessary to brush twice daily for atleast two weeks to see reductions in dentin hypersensitivity, and for four to eight weeks to demonstrate significant relief as compared to regular fluoride toothpaste [6].

Traditionally both in-office and over-the-counter products have been used to treat sensitivity usually by occluding the open dentinal tubules on the exposed root surface. There is debate as to the effectiveness of desensitizers containing ions which act on the pulpal nerves as they must migrate inwards against the direction of flow of the dentinal fluid to reach their point of operation [7].Therefore, the majority of current research is based on the concept of permanently blocking the dentinal tubules thus reducing flow and sensitivity. However, there appears to be no ideal material which permanently occludes dentinal tubules. Active ingredients for occluding the dentinal tubules include stannous fluoride, strontium chloride, hexahydrate, and aluminum, potassium or ferric oxalates and fluorides. Other active agents that have been proven to be effective as a desensitizing agent are dentin sealers (resins), sodium citrate, and sodium monofluorophosphate [8,9]Among these, strontium chloride has been largely used for this purpose, and previously published studies have shown that strontium chloride can effectively occlude the open tubules.

Dentifrices containing 10% strontium chloride hexahydrate as the desensitizing agent have been widely available for four decades. The incorporation of strontium chloride in a dentifrice has enjoyed success, some trials claiming 75 - 80% improvement [10]. According to a study done by Minkoff [11]therapeutic response to the strontium chloride as an active agent was apparent within 2 weeks and increased continuously thereafter. Recently, a newer formulationin the form of desensitizing dentifrice containing the amino acid arginine was introduced [12].Based on arginine, bicarbonate, and calcium carbonate, its utility both as an anticaries and desensitising agent has previously been claimed [13].It is reported to work through binding of positively charged agglomerates to exposed dentine surfaces and within the tubules themselves.Multiple independent clinical studies have claimed that the 8% arginine and calcium carbonate (pro argin technology) delivers "immediate" relief of dentin hypersensitivity following a single direct topical application [14].

In the present study, the effect on dentinal tubule occlusion of a new dentifrice formulation based on Pro-argin technology was compared withdentifrice formulation containing strontium chloride, after one time applicationthrough scanning electron microscope.

Methods: Before beginning the study ethical approval was taken from the institutional ethical committee of the college and written informed consent was taken from all the participants.

Specimen preparation: Orthodontically extracted fresh human premolars without caries were collected and stored in formalin solution. The teeth were sectioned horizontally at the cemento-enamel junction using a diamond wheel disc, and the dentin in the root portion was used for investigation. The dentin specimens were treated with EDTA for 2 min to completely open the dentinal tubule and ultrasonicated in distilled water for 10 minto remove the residual smear layer. The specimenswere placed in distilled water until they were required for treatment.

Experimental groups: The specimens were randomly divided into the following three groups, each containing twenty specimens; group 1:distilled water (control), group 2: 10% strontium chloride based dentifrice (Thermoseal, ICPA Health Products Ltd., GIDC, Ankleshwar, Gujarat, India)) and group 3: 8% arginine + calcium carbonate (Colgate sensitive pro-relief (Colgate Palmolive (India) Ltd., Mumbai, Maharashtra, India)).

Treatment and analysis: Dentifrice slurries were prepared as 20 g of dentifrice in 80 mL of distilled water. This was applied to the specimen with a brush. Tooth-brushing was performed at a force produced by 150 g weight attached to the brush head and at a rate of 50 back and forth strokes per minute. The specimen were then rinsed with distilled water and dried.

After drying, each specimen was mounted onto stubs with conducting carbon cement. Specimens were mounted flat for surface views. They were then sputter coated with gold for SEM. In this study Scanning electron microscope (JSM-5610 LV,JEOL Ltd., Tokyo, Japan) was used to observe the surface of all specimens. Micrographs were taken from randomly selected fields in the central portion of each disc at varying magnifications (3000 X). (figure 1, 2 and 3)A quantitative analysis was done. The specimens of each group were designated for occlusive percentage calculation of the tested material on the orifices of dentinal tubules. Occlusive percentage was defined as the number of the orifices of dentinal tubules occluded by tested material divided by the total number of the orifices of dentinal tubules at × 3000 magnification.

By virtue of image analyzer software mean of diameter of 20 open tubules selected randomly form each specimen control group was calculated (M = 1.1 micron). Three categories were formed on the basis of occlusion of tubulescompletely occluded, partially occluded and open tubules. Completely occluded tubules were defined as the tubules which showed either complete obliteration of tubules or 90% reduction in tubule diameter compared to M (M≥0.99μ). Partially occluded tubules were defined as the tubules which showed 89-25%reduction in tubule diameter compared to M (M≤0.99 and ≥0.275μ) and Open tubules were defined as the tubules which showed 24%or less than reduction in diameter compared to M(M≤0.275μ). All the analysis of tubule diameter was done through in built image analyzer software.

One micrograph per specimen was considered for all the groups.Each micrograph was individually assessed for occlusive percentage of completely, partially occluded and open tubules. Mean of all the occlusive percentages of completely, partially occluded and open tubules were calculated for each group.

Results were drawn under three sections of completely occluded, partially occluded and open tubules. Results of all three groups were than statistically analysed and compared under these sections.

Statistical Analysis: The data was coded and entered into Microsoft Excel spreadsheet. Analysis was done using SPSS version 15 (SPSS Inc. Chicago, IL, USA) Windows software program. Measure of central tendency using mean was calculated, and measure of spread using standard deviation and range were also calculated. Level of confidence interval and p value was set at 95% and 5% respectively.

Results: Occlusive percentage of each specimen under different sections was calcuated. All the values were added up and mean was derived. As per graph 1, it is clear that group 3 i.e. 8% arginine and calcium carbonate group shows highest complete tubule occlusion, with a statistically significant difference from group 1 and group 2.(table 2)

Both group 2 and group 3 show statistically significant improvement for partially occluded tubules as compared to group 1, but the difference in between group 2 and group 3 is not statistically significant.(table 3)

Similarly, for open tubules, both group 2 and group 3 show statistically significant improvement from group 1, but the difference in between group 2 and group 3 is not statistically significant.(table 4)

Table: 1

	Completely occluded	Partially occluded	Open
Group 1	7.5%	18.6%	73.8%
Group 2	49.9%	37.3%	10.9%
Group 3	59.06%	32.9%	7.9%

For Completely Occluded Tubules
Table 2: Post-hoc ANOVA

(I) VAR00003	(J) VAR00003	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-15.050*	.638	.000	-16.62	-13.48
	3.00	-20.450*	.638	.000	-22.02	-18.88
2.00	1.00	15.050*	.638	.000	13.48	16.62
	3.00	-5.400*	.638	.000	-6.97	-3.83
3.00	1.00	20.450*	.638	.000	18.88	22.02
	2.00	5.400*	.638	.000	3.83	6.97

*. The mean difference is significant at the .05 level.

Table 3: Post-hoc ANOVA

(I) VAR00003	(J) VAR00003	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-6.400*	.595	.000	-7.87	-4.93
	3.00	-5.700*	.595	.000	-7.17	-4.23
2.00	1.00	6.400*	.595	.000	4.93	7.87
	3.00	.700	.595	.733	-.77	2.17
3.00	1.00	5.700*	.595	.000	4.23	7.17
	2.00	-.700	.595	.733	-2.17	.77

*. The mean difference is significant at the .05 level.

Mean Diameter Of All The Partially Occluded Tubules In Each Group Was Also Calculated
For Open Tubules
Table: 4 Post-hoc ANOVA

(I) VAR00003	(J) VAR00003	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	26.700*	.621	.000	25.17	28.23
	3.00	26.200*	.621	.000	24.67	27.73
2.00	1.00	-26.700*	.621	.000	-28.23	-25.17
	3.00	-.500	.621	1.000	-2.03	1.03
3.00	1.00	-26.200*	.621	.000	-27.73	-24.67
	2.00	.500	.621	1.000	-1.03	2.03

*. The mean difference is significant at the .05 level.

Figure 1: Control group at X 3000 magnification

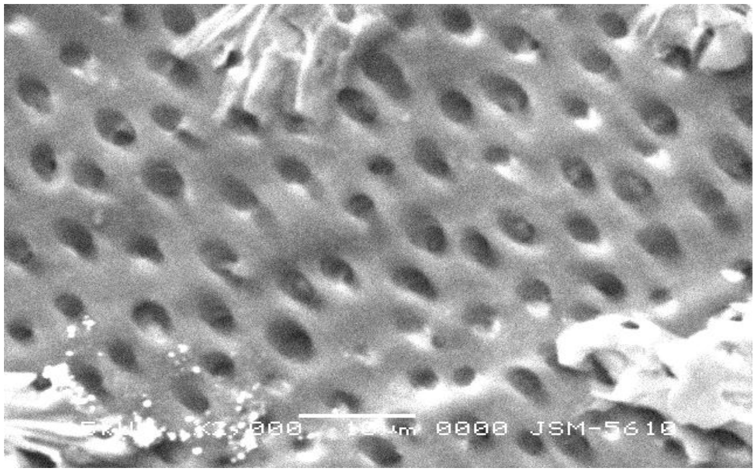


Figure 2: Strontium chloride group at X 3000 magnification

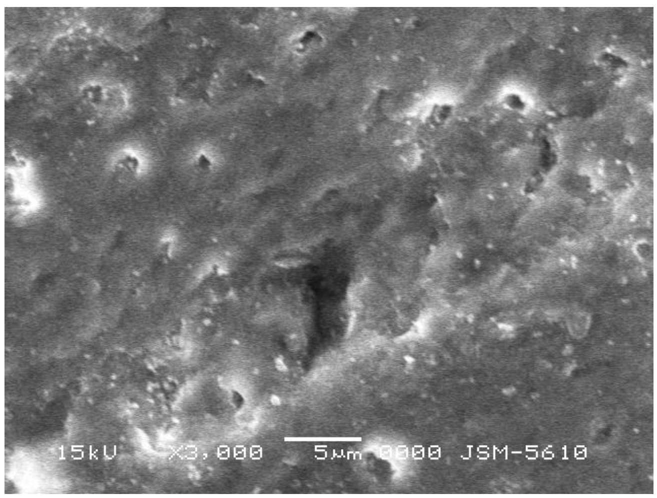
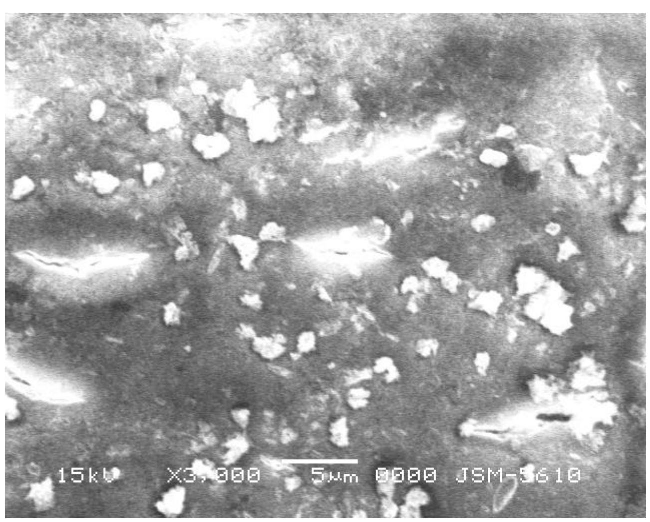
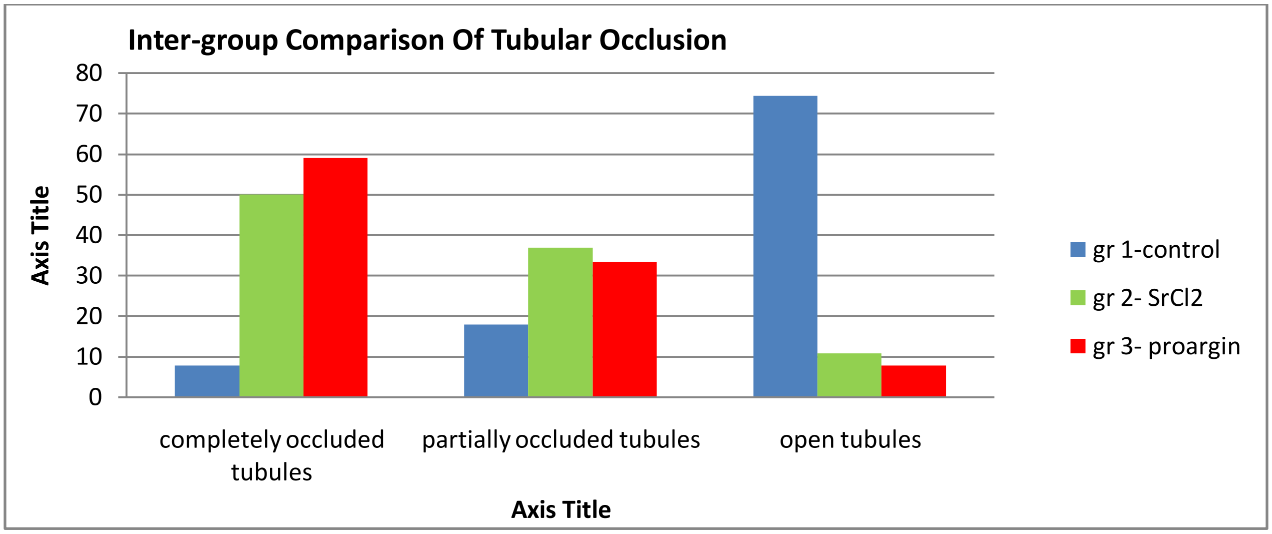


Figure 3: Proargin group at X 3000 magnification



Graph 1



Discussion: In the present study, the dentine disc model was employed to examine the tubule-occluding properties of the desensitizing agents as it represents close approximation to the in vivo situation. Absi et al [15] have reported on a positive relationship between tubular patency and sensitivity. Scanning electron microscopy was used to provide a qualitative indication of the degree of tubule occlusion on the exposed dentine surface in dentine disc model.

The cervical area of the premolars i.e. at the cemento-enamel junction was analysed because it was more appropriate (higher number of dentinal tubules) according to Kerns et al [16] strontium chloride is one of the many desensitizing agents that have been used to treat sensitivity with varying degrees of success. Lower reduction in sensitivity by strontium chloride may be due to its mechanism of action Studies by Henry O Trowbridge et al [17] showed that strontium strongly absorbs to calcified tissues. It has been suggested that strontium deposits are produced by an exchange with calcium in the dentin, resulting in recrystallization in the form of a strontium apatite complex [18, 19]. This type of precipitation is known to reduce the diameter of the open tubules.

Dentifrices containing strontium chloride have been available since quite some time. Various clinical and microscopic studies have been conducted to test and prove its efficacy. While the weight of evidence supports it use, the degree of efficacy of strontium compared to other agents and the duration of its effect have been considered uncertain by some authors [20].

According to a study by Oberg et al strontium chlorideresulted in high dentin permeability, with opened and partially obliterateddentin tubules visible through SEM analysis [21].

Another dentifrice used in our study was Colgate sensitive pro-relief based on Pro-argin technology. The Pro-Argin technology consists of arginine, a naturally occurring amino acid, and an insoluble calcium compound, in the form of calcium carbonate. These ingredients are delivered in a paste containing a mild abrasive system, and can be used on teeth exhibiting dentin hypersensitivity. Mechanism of action studies have shown that this technology physically seals dentin tubules with a plug that contains arginine, calcium, and phosphate. This plug, which is resistant to normal pulpal pressures and to acid challenge, effectively reduces dentin fluid flow, and thereby reduces sensitivity. Clinical studies have demonstrated that a toothpaste based on Pro-Argin technology, i.e. containing 8% arginine, calcium carbonate, and 1450 ppm fluoride provides rapid and lasting reductions in dentin hypersensitivity and superior sensitivity relief when burnished directly onto a site that is sensitive, and that sensitivity relief experienced following this single application is long lasting [22-23].

In early research studies, Kleinberg proposed that the combination of arginine and calcium carbonate acts by forming a plug that occludes the dentin tubules. He suggested that positively charged arginine is attracted to the negatively charged dentin surface where it helps attract and adhere calcium carbonate to the dentin surface and deep into the tubules. The association of arginine and calcium carbonate in situ provides an alkaline environment which encourages endogenous calcium and phosphate ions to deposit and further plug and occlude the dentinal tubules [24-26].The SEM analysis by Petrou et al [13] shows that treatment with prophylaxis paste containing 8% arginine and calcium carbonate was highly effective in occluding dentin tubules. The in-office desensitizing paste with Pro-Argin technology has been clinically proven to provide instant sensitivity relief when applied with a prophy cup after professional cleaning procedures, and that the benefit of a single treatment lasts for at least 28 days [14].

In the present study, we have calculated both partially occluded and completely occluded tubules. For a material to be more effective and provide immediate relief it has to completely occlude the tubules, while causing more partially occluded tubules will signify that the material will need further application for causing completely occluded tubules and will not provide immediate relief. Both the groups showed created a smear layer on the dentin surface that significantly reduced the diameter of dentin tubules after treatment (figure 3 and 4). But the results also show that there is statistically significant difference between proargin and strontium chloride group in causing more number of complete tubule occlusion amounting to 59% from that of strontium chloride which shows only 49% (table 1).The amount of partial occlusion as well as open tubules caused by the proargin group and strontium chloride appeared to have no statistically significant difference. Thus suggesting that on inital application proargin technology is more effective in complete tubular occlusion which would result in effective relief from dentinal hypersensitivity and on further application could provide better results.

Conclusion: Proargin group caused more completely occluded tubules than strontium chloride group and the difference was statistically significant, whereas there was no statistically significant difference in partially occluded tubules. Thus proargin technology appears more promising in providing immediate relief as well as could be more effective on further application.

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