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Evaluation of Dentinal Tubule Occlusion Using Three Different Desensitizing Dentifrices – An SEM Analysis

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Abstract

Objective: The aim of this study was to evaluate three different desensitizing tooth pastes containing Bioactive glass (Biomin) and Potassium nitrate and Fluoride (control) on dentinal tubule occlusion before and after surface treatment with citric acid.

Materials and methods: A total of 45 dentin specimens with patent tubules were randomly divided into 3 groups. Group 1 –Biomin; Group 2 –Potassium Nitrate; Group 3 – Fluoride dentifrice was used respectively for brushing of specimens. Each group was divided into 3 subgroups based on the surface treatment with citric acid and artificial saliva as follows: Subgroup A (1,2,3) – directly underwent scanning electron microscope analysis; Subgroup B (1,2,3) – soaked in 0.3% citric acid for 5 minutes followed by SEM analysis; Subgroup C (1,2,3) – soaked in artificial saliva for 5 minutes followed by SEM analysis. Percentage of tubule occlusion from each group was analyzed. Statistical analysis was done using one-way anova, Tukey's HSD post hoc test.

Results: Biomin containing dentifrice group showed more tubule occlusion compared to other two dentifrices.

Keywords: Biomin, Potassium Nitrate, Fluoride, Citric acid, Artificial saliva, Scanning electron microscope, Dentin hypersensitivity.

Introduction

Dentin hypersensitivity (DH) is a short sharp pain arising from exposed dentin in response to various stimuli like thermal, evaporative, tactile, osmatic or chemical and which cannot be ascribed to any other form of dental defect or pathology. [1]

Excessive consumption of dietary acids such as citrus juices, carbonated drinks, wines, and ciders have been identified as the potential risk factors for dental hypersensitivity.[2]

Removal of the enamel covering the crown of the tooth or denudation of the root surface occurring due to loss of the cementum secondary to a disease process are the main etiologies causing exposure of dentin that culminate in dentin hypersensitivity.[1]

According to Dr. Gilliam, '33% of the population may be affected,' 'but for the majority it is a relatively minor and episodic or transient problem. However, for about 10% of the general population it may be a severe problem and have a major impact on their quality of life.' Dr. Gillam suggested that DH can present at any age, but the majority of sufferer's range between 20-50 years, with a peak prevalence at the age of 30-39 years.

The teeth most commonly affected are premolars and canines, particularly on the buccal aspect due to over-zealous and/or incorrect brushing, but all teeth could potentially be involved and some recent studies have shown molars to be affected. 'Cold food and drink, and cold air seem to be the most common triggers'. 'There are other triggers, such as hot foods, but they don't appear to have the same effect as cold on dentin hypersensitivity.'[3]

According to the hydrodynamic theory, there are two methods for treating DH: 1) coating the dentin surface and blocking the tubules or 2) desensitizing the pulpal nerves that are responsible for pain.

Different products such as fluorides, oxalates, varnishes, adhesive resins, sealants, lasers, Portland cement and Bioglass, have been tested for blocking open dentinal tubules on the root surface. Most current research is focused on reduction of dentin permeability via tubule occlusion as an important strategy for treating sensitive teeth. Nevertheless, reducing dentin permeability can also prevent activation of intradental nerves.[4]

A bioactive glass, containing Fluoro calcium phosphosilicate, also known as Biomin was introduced in 2016. It contains fluoride, and higher phosphate content, besides having a smaller average particle size. Upon contact with the exposed tubules, it forms fluorapatite.[5]

Toothpaste formulations containing potassium designed to treat sensitive teeth can also have an analgesic effect. Potassium nitrate has been postulated to act by blocking neural transmission to reduce DH symptoms.[6]

Potassium salts act by diffusion along the dentinal tubules and decreasing the excitability of the intradental nerve fibers by blocking the axonic action. Various clinical studies have shown the efficacy of potassium salts in controlling the DH.[7]

Fluoride containing dentifrices are as effective as the conventionally formulated dentifrice in the treatment of DH. The efficacy of these dentifrices has been evaluated in previous studies. Hence, it was chosen as the control toothpaste.

In an attempt to produce better adherence of the constituents of desensitizing dentifrices which will reduce the reopening of dentinal tubules, this study was undertaken using biomin, potassium nitrate and fluoride dentifrice as a control on dentinal tubule occlusion before and after an acid challenge and immersion in artificial saliva.

Materials and Methods

Forty-five dentin discs specimens were obtained from permanent human molar teeth. The effects of two dentifrices, containing BioMin (Elsenz® Group Pharmaceuticals Ltd., India) and Potassium Nitrate (Thermoseal RA) together with a control dentifrice containing Fluoride (COLGATE Total® Colgate-Palmolive Pvt. Ltd., India) on dentin tubule occlusion were assessed by scanning electron microscopy (SEM).

Preparation of dentin specimens

Extracted permanent human third molar teeth which underwent surgical extractions were stored in normal saline. Teeth free from periodontal disease, carious lesion, teeth with restorations and endodontic treatment, crown fracture, attrition, abrasion, erosion, external resorption, and developmental anomalies were selected. The collected teeth were debrided thoroughly to remove any remaining debris, and periodontal remnants using ultrasonic scaler.

Twelve dentin discs with a thickness of approximately 1 mm were obtained by placing cuts perpendicular to the long axis of the tooth from the region between the apical limit of the dentino-enamel junction and the coronal limit of the pulp chamber with a slow speed diamond disc. On an average 2 – 3 specimens were obtained from each tooth. The surface of each dentin disc was polished with a 600-grit silicon carbide paper for 30 s using back and forth motion. The smear layer was removed by immersing all the dentin discs in 6% citric acid for two minutes. The discs were removed from the citric acid solution and were immersed in de-ionized water for 30 seconds. Dentin discs were then fractured longitudinally using dental pliers in four quadrants to obtain 48 discs specimens.45 discs specimens were then mounted on the paraffin wax blocks to receive one of the three desensitizing agents. The forty-five specimen discs were randomly divided using computer generated randomization using SPSS software into three groups: Groups I, II and III each group comprising of fifteen specimens as follows:

Group 1 (n=15): Specimens were treated with a BioMin® containing dentifrice

Group 2 (n=15): Specimens were treated with a potassium nitrate containing dentifrice

Group 3 (n=15): Specimens were treated with a fluoride containing dentifrice

Treatment regimen

All specimens of Groups 1, Group 2 and Group 3 were gently rinsed using a drop of sterile saline following which a dab-on application of BioMin, Potassium nitrate or Fluoride containing dentifrice dispensed on brush of the undiluted respective dentifrice with circular motion using a powered toothbrush (Oral-B Pro-Health Precision Clean Electric Toothbrush) of circular head for two minutes.

Following application of respective dentifrice, the specimens in each group were further divided into three subgroups A, B and C consisting of five specimens each. Specimens from all subgroups were immersed in a jar filled with distilled water and stirred for one min with a plastic stirrer to ensure removal of any excess desensitizing agent. The discs in each group were treated in the following manner

Subgroup 1A: Specimens treated with BioMin containing dentifrice

Subgroup 1B: Specimens treated with BioMin containing dentifrice followed by citric acid challenge

Subgroup 1C: Specimens treated with BioMin containing dentifrice followed by artificial saliva immersion

Subgroup 2A: Specimens treated with potassium nitrate containing dentifrice

Subgroup 2B: Specimens treated with potassium nitrate containing dentifrice followed by citric acid challenge

Subgroup 2C: Specimens treated with potassium nitrate containing dentifrice followed by artificial saliva immersion

Subgroup 3A: Specimens treated with fluoride containing dentifrice

Subgroup 3B: Specimens treated with fluoride containing dentifrice followed by citric acid challenge

Subgroup 3C: Specimens treated with fluoride containing dentifrice followed by artificial saliva immersion

Subgroup 1A, 2A, and 3A directly underwent scanning electron microscope analysis.

Citric acid challenge

Specimens from subgroup 1B, 2B and 3B were then immersed in a jar containing 0.3% citric acid with sodium hydroxide buffer (NaOH) with pH of 5 for five minutes. Following a citric acid challenge, they were immersed in a jar of de-ionized water and stirred for one minute to ensure removal of any excess citric acid and were lightly dried using an air blast.

Artificial saliva treatment

Specimens from subgroup 1C, 2C and 3C were then immersed in a jar containing artificial saliva (Wet Mouth®) (simulating the oral environment) for five minutes, they were immersed in a jar of deionized water and stirred for one min to ensure removal of any excess of saliva and were lightly dried using an air blast.

Preparation of the specimens for SEM analysis

Dentin discs specimens were dried in the desiccator (under an infrared lamp for additional 15 minutes) and then later mounted on to aluminum stubs using a conductive carbon tape. These were then sputter coated with gold for further SEM analysis. Images were taken at magnifications of 2500x and 5000x.

Assessment of tubular occlusion

The extent of tubule occlusion was assessed using an scanning electron microscope (Central Manufacturing Technology Institute (CMTI), BANGALORE). The images were taken at various magnifications (2500x and 5000x) and then assessed for level of tubule occlusion (on a scale of 1-5) for 2500x magnification, in accordance with the ranking system established below that visualizing the extent of occlusion (visual score).

1: Occluded (100% of tubules occluded).

2: Mostly occluded (75% of tubules occluded)

3: Equally occluded/unoccluded (50% of tubules occluded)

4: Mostly unoccluded (25% of tubules occluded)

5: Unoccluded (0%: no tubule occlusion).

Percentage of tubule occlusion (% OCT) was evaluated using the formula[1] for 5000x magnification:

% OCT=Number of occluded tubules × 100

% OCT= (Number of occluded tubules × 100) ÷ Total number of tubules

Statistical analysis:

Data was analyzed using the statistical package **SPSS 22.0** (SPSS Inc., Chicago, IL) and level of significance was set at **$p<0.05$**. **Descriptive statistics** was performed to assess the mean and standard deviation of the respective groups. Normality of the data was assessed using **Shapiro Wilkinson test**. **Inferential statistics** to find out the difference between the groups was done by **Oneway ANOVA and Tukey's HSD posthoc test.**

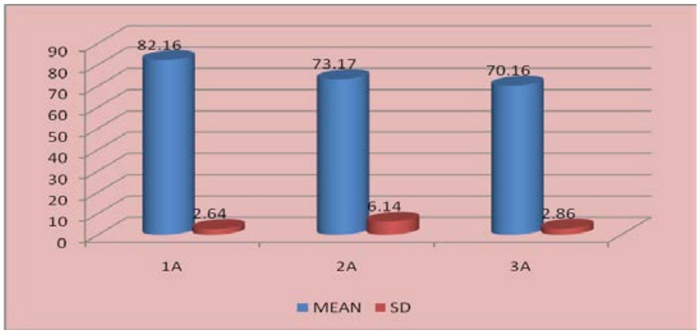
Results

Table 1: Comparison of mean dentinal tubule occlusion between the subgroups of groups 1, 2, 3.

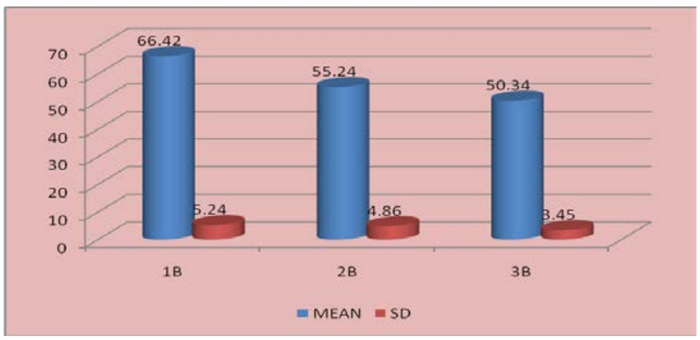
Subgroup	Mean±SD	P value
1A	82.16±2.64	<0.05*
2A	73.17±6.14	
3A	70.16±2.86	
1B	66.42±5.24	<0.05*
2B	55.24±4.86	
3B	50.34±3.45	
1C	79.26±1.99	<0.05*
2C	58.43±3.79	
3C	57.66±1.34	

The results of the present study showed maximum dentin tubule occlusion was observed in group 1 (Biomin group) than group 2 and group 3. There was significant difference between group 1 and group 2, group 1 and group 3 in all subgroups.

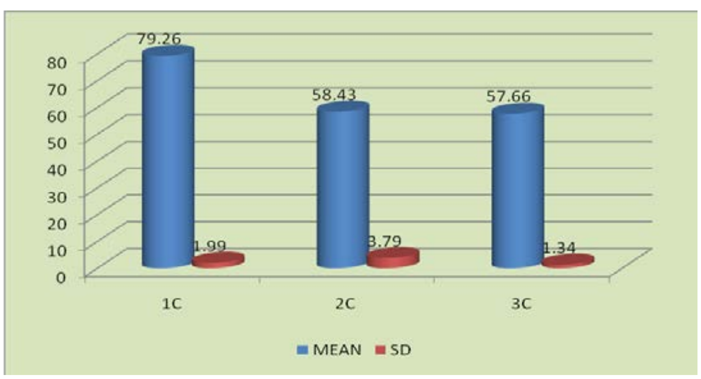
Graph 1- Dentinal tubule occlusion of subgroup A of group 1,2,3.



Graph 2 - Dentinal tubule occlusion of subgroup B of group 1,2,3.



Graph 3 - Dentinal tubule occlusion of subgroup C of group 1,2,3.



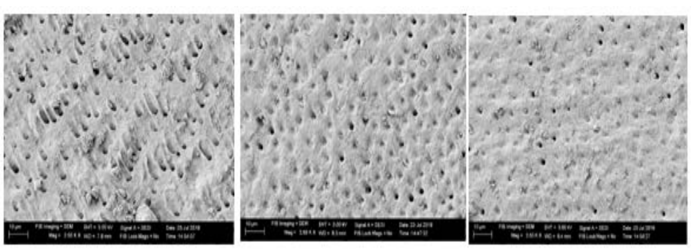


Figure: A **B.** **C**

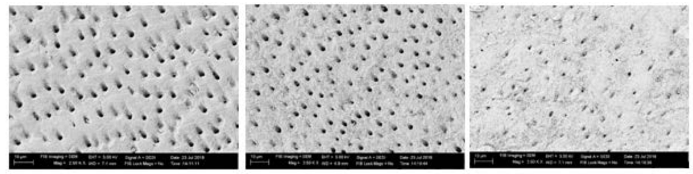


Figure: A **B.** **C**

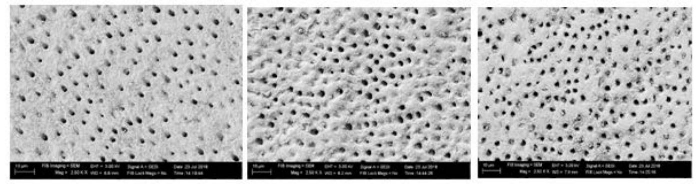


Figure: A **B.** **C**

Figure 1: Scanning Electron Microscope (SEM) Images Of Dentinal Tubule Occlusion Of Groups 1, 2, 3 And Subgroups A, B, C. SEM Images At 2500X.

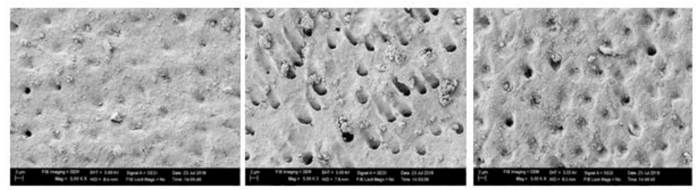


Figure: A **B.** **C**

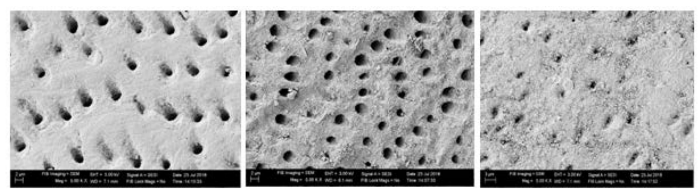


Figure: A **B.** **C**

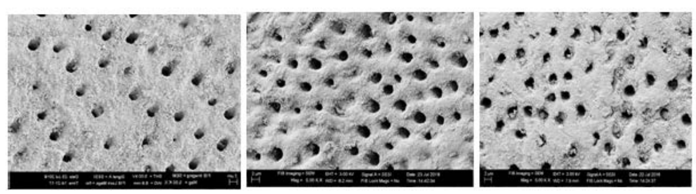


Figure: A **B.** **C**

Figure 2: Scanning electron microscope (SEM) images of dentinal tubule occlusion of groups 1, 2, 3 and subgroups A, B, C. SEM images at 5000X.

Discussion

Dentin hypersensitivity (DH) is a painful condition which arises when the dentin tubules become exposed and patent to the pulp due to etiological factors such as Abrasion, Abfraction, Erosion, Gingival recession, Quality of the buccal bone, Periodontal disease and its treatment, Surgical and restorative procedures, Patient destructive habits.[8,3]

The second most common dental condition of concern to patients is dentin hypersensitivity. Removal of cementum or enamel leaves the dentin exposed allowing various stimuli to produce fluid movement through the dentinal tubules.

The fluid movements are believed to activate the sensory nerves of the pulp, leading to pain. This pain is typically a short burst for a short span of time and has been claimed to be due to stimulation of A-beta and A-delta nerve fibers.[9]

The theory proposed by Brannstrom is the most widely accepted to explain the mechanism of pain in DH, one of the main strategies to treat this condition consists in sealing the dentinal tubules, thus preventing fluid flow.[10]

There are varieties of treatment regimens recommended over the years to cure dentin hypersensitivity. Home use dentifrices containing various active components which act by either blocking the hydrodynamic mechanism or the neural response has got more attention.

The aim of the present study was to compare the desensitizing effects of Biomin and potassium nitrate containing dentifrices with a fluoride containing dentifrice, following a citric acid challenge and immersion in artificial saliva.

With the findings of the present study, the Biomin containing dentifrice, showed better percentage of dentinal tubule occlusion compared to other dentifrices used.

Biomin is an inorganic, amorphous metal- derived biocompatible glass compound that contains

calcium, fluoride, phosphate, and silica. The active ingredient is the inorganic chemical fluoro calcium phosphosilicate. In addition to fluoride in the glass, it has three times higher phosphate content, which promotes fluorapatite formation and has lower silica and penetrates effectively into the dentinal tubules for occlusion.[11]

The increased phosphate content of Biomin aids both the effectiveness and the speed of re-mineralization, and as the calcium, phosphate and fluoride ions are released, these work in concert with the saliva in the mouth to restore equilibrium following acid attack, and form fluorapatite material, which is more stable and resistant to acid than hydroxyapatite.[3]

The results are in accordance with the study conducted by Pereira R et al[1]. A clinical study conducted by Reddy et al[11], for a period of 4 weeks concluded that Biomin has showed significantly better results, which are in accordance with the present study.

One of the approaches is to interrupt the neural response to pain stimuli by inserting potassium ions through the dentin tubules to the A-fibers of the nerves, thereby decreasing the excitability of these nerves. Potassium nitrate (KNO_3) is used to provide potassium ions to decrease the excitability of the nerves that transmit pain sensations.[12]

These ions have a direct impact on nerve excitability. By increasing the potassium ion concentration adjacent to the dentinal nerve terminals, there is depolarization and activation of nerve fibres. A prolonged period of depolarization results in inactivation of the action potential. Divalent cation solutions stabilize the nerve membrane without changing the membrane potential. potassium based toothpastes must be used for a minimum of 2 weeks, twice daily to bring about a reduction of sensitivity; and for a period of 4-8 weeks to demonstrate significant pain relief. The cited reasons for this are that the potassium ions must diffuse from the oral cavity into the dentinal tubules, and further against dentinal fluid flow to the site of action, which is the nerve endings. The concentration of potassium ions must also build up to significant quantities to achieve depolarization and pain relief, which takes 4-8 weeks. Furthermore, when the use of these pastes is stopped, the potassium ions at the site of action are diffused, and the relief of sensitivity is lost.[9]

In the present study, the potassium nitrate has showed significant tubular occlusion than the control group. When treated with citric acid, the dentinal tubules were open which may be due to the loss of mineral deposition. The results are in accordance with the studies done by Rahardjo et al.[12] and Wang et al[13].

A clinical study done by Saurabh S Prithyani., *et al.*[14] where Biomin, proarginine and potassium nitrate dentifrice were used concluded that the dentifrice containing fluoro calcium phosphor silicate (Biomin) is more efficacious in managing dentinal hypersensitivity.

The patent dentinal tubules are naturally occluded by saliva by transporting calcium and phosphate ions into them to induce tubule plugging and thus forming a surface protective layer of a salivary glycoprotein. However, this process of natural tubule occlusion is very slow, and the tubule plugging may be easily removed by both dietary acid and physical insult (e.g., tooth brushing) thus rendering it neither effective nor reliable in providing lasting relief of dentine hypersensitivity.[1] In the present in vitro study, the percentage of dentinal tubule occlusion in all three dentifrice groups treated with artificial saliva was not statistically significant with the specimens treated with Biomin and potassium nitrate dentifrices alone ,which underwent direct SEM analysis.

Considering the nature and limitations of the in vitro experimentation this study should be interpreted with caution before extrapolating them in dental practice. The desensitizing effect of these agents on vital teeth can be determined only in a clinical situation. The manual counting of dentinal tubules for calculating the percentage of tubule occlusion is subjective to human error. To evaluate the clinical effectiveness of the above mentioned treatment modalities of the present study, large scale, multicenter, randomized controlled in vivo clinical trials are required.

Conclusion

Within the limitations of this in vitro study it was observed that, the Biomin containing desensitizing agent has showed significantly better results compared with postassium nitrate in reducing dentin hypersensitivity.

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