Effects of Two Desensitizing Dentifrices on Dentinal Tubule Occlusion with Citric Acid Challenge: Confocal Laser Scanning Microscopy Study

Abstract

Background: Dentin hypersensitivity results when patent tubules are exposed to pain-inducing external stimuli. Aim: This study aims to compare the effects of two desensitizing dentifrices containing NovaMin and arginine on dentinal tubule occlusion with and without citric acid challenge in vitro using confocal laser scanning microscopy (CLSM). Materials and Methods: Forty dentin discs were randomly divided into Groups I and II containing twenty specimens each, treated with NovaMin and arginine-containing dentifrices, respectively. Groups I and II were divided into subgroups A and B where IA and IIA underwent CLSM analysis to determine the percentage of tubule occlusion while IB and IIB underwent 0.3% citric acid challenge and CLSM analysis. A novel grading system was devised to categorize tubule occlusion. Results: In Group II, the percentage of occluded tubules was highest for IIA (72.25% ± 10.57%) and least for IIB (42.55% ± 8.65%) having statistical significance (P < 0.0005). In Group I, the difference between IA (49.9% ± 12.96%) and IB (43.15% ± 12.43%) was statistically insignificant (P = 0.249). On the comparison between IB and IIB statistically indifferent result was obtained (P = 0.901), whereas the difference between IA and IIA was statistically significant (P < 0.001). The results of grading system were for IA 50% of samples belonged to Grade 2, for IIA 60% - Grade 3, and for IB 70% and for IIB 90% - Grade 2.

Conclusion: Dentinal tubule occlusion with arginine-containing dentifrice was significantly higher than NovaMin. However, it could not resist citric acid challenge as effectively as NovaMin. The effects of NovaMin were more sustainable as compared to arginine-containing dentifrice, thus proving to be a better desensitizing agent.

Keywords: Arginine, bioactive glass, citric acid, confocal laser scanning microscopy, dentine hypersensitivity

Introduction

Dentin hypersensitivity is characterized by short, sharp pain arising from exposed dentin in response to stimuli typically thermal, evaporative, tactile, osmotic or chemical, which cannot be ascribed to any other form of dental defect or pathology. It is a painful clinical condition affecting 8%–57% of the adult population worldwide and is characterized by dentin exposure to oral environment. It typically affects the individuals in the age range of 20–49 years, most commonly women at a younger mean age. The mechanism of the external stimulus, leading to the pain of dentin hypersensitivity, is explained aptly by hydrodynamic theory given by Gysi in 1900 and later scientifically explained by Brännstrom in 1966. Briefly, this theory suggested that due to the hydrodynamic fluid shifts occurring across the exposed dentin with open tubules, mechanical activation of the nerves situated at the inner ends of the dentinal tubules or in the outer layers of the pulp occurs, leading to the symptoms of dentin hypersensitivity.

More specifically, the two main approaches of treating dentin hypersensitivity include interference of nerve transmission and occlusion of the open dentinal tubules. Despite the numerous clinical trials and research of the clinicians worldwide, the holy grail for the treatment of dentin hypersensitivity is yet to be defined. Formulations containing potassium nitrate, oxalates, strontium-based compounds, citrate-based compounds, stannous fluoride, etc., as active ingredients in the form of dentifrices and mouth rinses are available as over-the-counter desensitizing medications that are routinely used by the masses. One of the major disadvantages of the conventional treatment modalities is that they have to be...
repeated regularly to achieve continuous and long-lasting pain relief.[9] Hypersensitivity usually reappears due to toothbrush abrasion, the presence of acid challenges in the mouth, and/or degradation of the coating material.[8]

Recent additions to the superfluity of active ingredients in dentifrices are calcium sodium silicon phosphate (NovaMin®) and arginine which act by mechanical occlusion of dentinal tubules. Bioactive glass is made of bioactive glass-ceramic (e.g., Bioglass®) which has been ground into a fine powder with particulate size of <20 μm, is the key ingredient in NovaMin®,[7,8] and has been shown to physically occlude dentinal tubules.[8,9] The key components of NovaMin® are silicon dioxide, sodium oxide, calcium oxide, and phosphorus pentoxide. NovaMin® has been shown in vitro and in vivo to reduce sensitivity by blocking open tubules and by supplying calcium (Ca²⁺) and phosphate (PO₄³⁻) ions in an aqueous environment to form hydroxyapatite.[7]

Several decades of research by Kleinberg and SensiStat have led to the development and validation of a new in-office treatment of dentin hypersensitivity based on 8% arginine, an amino acid present naturally in saliva, bicarbonate, a pH buffer, and calcium carbonate.[9] The essential components of this technology are arginine, an amino acid which is positively charged at physiological pH, i.e., pH 6.5–7.5, bicarbonate, which is a pH buffer, and calcium carbonate, which is a source of calcium.[10]

Dietary acids are a common part of the modern diet, particularly fruit acids. There is strong evidence that the manner in which the acidic food or drink is consumed is more important than the overall quantity.[10] Holding, swishing, or retaining acidic drinks in the mouth prolongs the acid exposure on the teeth increasing risk of erosion and also increases their susceptibility to mechanical wear. 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.[7] Therefore, there is a need for a material that will chemically react with the surface of dentin, will intimately adhere to tooth structure, and will significantly reduce the possibility of reopening dentinal tubules due to prolonged contact with oral fluids, eating of acidic foods, and/or vigorous tooth brushing.

The aim of the present study was to compare the effects of two desensitizing dentifrices containing NovaMin and arginine on dentinal tubule occlusion with and without acid challenge evaluated using confocal laser scanning microscopy (CLSM).

Materials and Methods

This in vitro study was performed on 40 dentin discs obtained from permanent premolar and/or molar teeth at MGM Dental College and Hospital, Navi Mumbai, India. This study was conducted from March 2014 to December 2014 in the Department of Periodontics at MGM Dental College and Hospital, Navi Mumbai, India. The protocol was approved by the Internal Review Board. The study purpose was explained, and informed consent was obtained from subjects whose extracted teeth were used for the study. The effects of two commercially available dentifrices containing NovaMin (SHY-NM™, Group Pharmaceuticals Ltd., Karnataka, India) and arginine (COLGATE® SENSITIVE PRO-RELIEF™, Colgate-Palmolive Pvt. Ltd., Solan, Himachal Pradesh, India) on dentin tubule occlusion were assessed by CLSM analysis with and without citric acid challenge.

Preparation of dentin specimens

Extracted permanent premolar and/or molar teeth which underwent surgical extraction and/or extraction for orthodontic reasons were obtained on a daily basis and were stored in normal saline for 1 month. 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 debris, and periodontal remnants using the ultrasonic scaler (Satalec®, Acteon Group, Gustave, France) and the root surfaces were planed using curettes (Gracey curettes 1–2, Hu-Friedy, Chicago, IL, USA) to remove the tissue remnants. After debridement, the teeth were stored in 0.5% thymol for a period not more than 1 month before using them for the preparation of dentin discs. 40 dentin discs with a thickness of approximately 600 μm–1 mm were obtained by placing cuts perpendicular to the long axis of the tooth from the region between the apical limit of the dentinoenamel junction and the coronal limit of the pulp chamber based on Pashley’s dentin-disc mode[11] using diamond disc (22 mm diameter) (SS White, Lakewood, NJ, USA). On an average, two to three usable 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 0.5M ethylenediaminetetraacetic acid (EDTA) solution in a petri dish which was subjected to ultrasonic vibration (Sonicator® Qsonica, LLC, Newtown, CT, USA) for 2 min. The discs were removed from the EDTA solution and were immersed in deionized water for 30 s. The discs were then mounted on the paraffin wax blocks to receive one of the two desensitizing agents.

The forty specimen discs were randomly divided into two Groups I and II, using coin toss method, each comprising of twenty specimens each: Group I (n = 20): Specimens were treated with NovaMin containing dentifrice, Group II (n = 20): Specimens were treated with arginine-containing dentifrice.
Treatment regimen

All discs of Groups I and II were wetted using a drop of sterile saline following which a dab-on application of NovaMin or arginine-containing dentifrice dispensed on toothbrush (Colgate Kids Plus, Colgate®, Mumbai, India) with a small head and medium bristle thickness was used in circular motion for 1 min. After this, the specimens in each group were further divided into two subgroups A and B consisting of ten specimens each. All specimens from subgroups IA and IIA were not subjected to citric acid challenge whereas specimens from subgroup IB and IIB underwent a 0.3% citric acid challenge for 5 min.

Citric acid challenge

Specimens from subgroups IB and IIB were immersed in a plastic jar filled with distilled water and stirred for 1 min with a plastic stirrer to ensure removal of any excess desensitizing agent. These specimens were then immersed in a petri dish containing 0.3% citric acid (LabChemie Pvt. Ltd., Mumbai, India) with a sodium hydroxide buffer (NaOH) with pH of 3.2 for 5 min. Following citric acid challenge, they were immersed in a jar of deionized water and stirred for 1 min to ensure removal of any excess citric acid and were allowed to air dry.

Assessment of tubular occlusion

All specimens from subgroups IA, IIA, IB, and IIB were completely dried, and viewed under CLSM (Zeiss LSM 510 META microscope and dual line argon-ion laser, ACTREC, Navi Mumbai, India) in a reflectance mode equipped with a 40X/1.3 oil immersion lens and a ×4 optical zoom to determine the tubule occlusion achieved with individual agent. Each photomicrograph was viewed for tubules that were occluded or empty as observed in the field of view at an optical zoom of ×4. The percentage of tubule occlusion for all groups was obtained by dividing the number of occluded tubules with the total number of tubules in the same field of view. This result was then multiplied by 100 to obtain the percentage of tubule occlusion for each photomicrograph. A novel grading system was devised to categorize the percentage of occluded tubules for all subgroups as shown in Table 1.

Statistical analysis

The statistical analysis was performed using the package for social services 16.0 for Windows (SPSS Chicago, IL, USA). The data collected was expressed as percentage of occluded tubules (%) and mean ± standard deviation calculated for subgroups IA, IIA, IB, and IIB. The distributions of percentage of occluded tubules were checked for normality using the Kolmogorov–Smirnov test. As the data followed a normal distribution, one-way ANOVA was carried out at 5% level of significance to test the equality of means of the groups with respect to percentage of tubule occlusion.

Results

Table 2 represents the descriptive statistics for the percentage of tubule occlusion (%) for subgroups IA, IIA, IB, and IIB. As shown in Table 2, the mean value of percentage of occluded tubules for IIA (72.25% ± 10.57%) was higher as compared to IA (49.9% ± 12.96%) which was statistically significant \( p < 0.001 \), Table 2 and Figure 1], whereas for IB (43.15% ± 12.43%) and IIB (42.55% ± 8.65%) did not show any statistical significance \( p = 0.901 \), Table 2 and Figure 1]. Furthermore, the mean value of IA (49.9% ± 12.96%) was higher as compared to IB (43.15% ± 12.43%) but did not show any significance \( p = 0.249 \), Table 3 and Figure 1]. The mean value of subgroup IIA (72.25 ± 10.57%) was higher than that of IIB (42.55 ± 8.65%), and it was statistically significant \( p < 0.0005 \), Table 3 and Figure 1].

The percentages of occluded tubules for subgroup IA, IIA, IB, and IIB were quantitatively categorized according to a new devised grading system. For the subgroup IA, 50% of

| Grade | Category (%) | Mean±SD (%) | p         |
|-------|--------------|-------------|-----------|
| 1     | 0–<25        | 49.9±12.96  | <0.001*   |
| 2     | 25–<50       | 72.25±10.57 | <0.0005   |
| 3     | 50–<75       | 43.15±12.43 | 0.901 (NS) |
| 4     | 75–100       | 42.55±8.65  |           |

\*p<0.05, statistically significant. NS=Not significant, SD=Standard deviation.

Table 3: Descriptive statistics showing the mean values, standard deviation of percentage of occluded tubules (%) for subgroups IA, IIA, IB, and IIB and results of one-way ANOVA for subgroup IA and IIA; IB and IIB

| Subgroup | Mean±SD (%) | p         |
|----------|-------------|-----------|
| IA       | 49.9±12.96  | 0.249 (NS) |
| IB       | 43.15±12.43 |           |
| IIA      | 72.25±10.57 | <0.0005*  |
| IIB      | 42.55±8.65  |           |

\*p<0.05, statistically significant. NS=Not significant, SD=Standard deviation.
the samples belonged to Grade 2 (25%–<50%); followed by 40% in the Grade 3 category and only 10% in Grade 4 [Figure 2]. For the subgroup IIA, 60% of the samples belonged to Grade 3 (50%–<75%); whereas 40% of the samples belonged to Grade 4 (75%–100%) [Figure 2]. For subgroup IB, 70% of the samples belonged to Grade 2 (25%–<50%); whereas 30% of the samples belonged to grade 3 (75%–100%) [Figure 3]. For subgroup IIB, 90% of the samples belonged to Grade 2 (25%–<50%), followed by 30% in the Grade 3 category [Figure 3].

Discussion

Dentin hypersensitivity has long been a dilemma for both dentists and patients. Several methods and materials such as varnishes, restorative materials, liners, dentinal adhesives, dentifrices, mouthwashes, iontophoresis, and lasers have been used as in-office methods to reduce dentin hypersensitivity. Furthermore, the effects of dietary acids have known to amplify the symptoms of dentin hypersensitivity. Although there are large number of techniques and therapeutic alternatives available in the literature, no single or combined modality has proven to be the gold standard till date. The present study compared the tubule occluding capacity of two desensitizing dentifrices containing NovaMin and arginine in dentinal tubule occlusion with and without citric acid challenge in vitro evaluated by CLSM.

In this study, the dentin discs were prepared based on the model proposed by Reeder et al.[11] The dentin disc model has been used extensively as a model for assessing the surface deposition and tubule occluding effects of desensitizing agents as well as the effects of these agents on fluid flow.[13] Most studies on tubule occlusion have focused on coronal dentin, where important variables such as dentin surface area, thickness and surface characteristics can be controlled.[14] Radicular and cervical dentin blocks have also been used for studying the occluding effects of desensitizing agents in vitro, but the mean number of tubules in the middle part of the root is significantly lower than in the middle part of the crown.[14] The dentin discs were prepared using a diamond disc and were subjected to polishing with a 600 grit polishing paper for 30 s which resulted in the formation of smear layer. Eick et al.[15] described smear layer as being composed of a crystalline phase embedded in an organic film with sulfur, carbon, and nitrogen. These smear plugs are composed of grinding debris whose particle sizes are <0.5–15[16] which are smaller than tubular orifices ranging from 2.4 to 2.9 μm.[16] Pashley[17] also showed that the smear plugs and the smear layer occupy 78.5% of the dentinal surface area and luminal area which affects the outcome of the evaluation of the microscopic photomicrographs. Hence, to remove smear layer all specimens were immersed in 0.5 M EDTA solution for 2 min.

As there exists no in vitro study comparing the desensitizing effects of NovaMin and arginine-containing dentifrice,
with the citric acid challenge, NovaMin containing dentifrice (43.15% ± 12.43%) [Figure 6] showed a greater percentage of occluded tubules compared to the arginine containing dentifrice (42.55% ± 8.65%) [Figure 7], but the difference was not statistically significant. The results demonstrated that NovaMin containing dentifrice at a concentration of 5% resisted the citric acid challenge better than arginine-containing dentifrice (Group I: 49% → 42%; Group II: 72% → 42%). Burwell et al.\(^\text{[18]}\) conducted a series of studies showing that a single application of NovaMin with a concentration of above 3%, either in a daily-use dentifrice or a professionally applied prophylaxis paste, was effective at blocking at least 75%–95% of open tubules. Furthermore, these studies also demonstrated that a single application of NovaMin in these models resisted repeated acid challenges.

In a study by Sauro et al.\(^\text{[19]}\) bioactive glass containing air-polishing powder completely occluded 90% of tubules which reduced to 47% after the citric acid challenge while arginine-containing air polishing powder showed 91% occluded tubules and reduced to 54% after the citric acid challenge. Bioactive glass created a dentin surface resistant to citric acid attack. Wang et al.\(^\text{[20]}\) evaluated the effectiveness of NovaMin after EDTA etching, initial application, 3-day application, 7-day application, and 6% citric acid challenge. A significant reduction in dentine permeability after the 7-day treatment was noted and showed excellent resistance to acid challenge under scanning electron microscopy was inferred. The better acid resistance of NovaMin containing dentifrice to acid challenge can be attributed to the release of sodium that allows rapid precipitation of particles and formation of an amorphous calcium hydroxyapatite layer.\(^\text{[21]}\) The rate of release of these ions has been well established and in fact, the earliest studies with this material demonstrated that a calcium hydroxyapatite layer could be formed \textit{in vitro}, even in the absence of an external source of calcium or phosphate.\(^\text{[21]}\)

The percentage of occluded tubules for the arginine containing dentifrice group without (72.25% ± 10.57%) and with (42.55% ± 8.65%) citric acid challenge showed a significant reduction in the percentage of occluded tubules.
Davies et al.\cite{21} also demonstrated a significant reduction in the percentage of occluded tubules by arginine-containing dentifrice as compared to strontium based dentifrice, following immersion in citric acid for 5 min, suggesting that occlusion by arginine is not stable following prolonged challenge with acid. Parkinsons\cite{22} compared the efficacy of strontium acetate and 8% arginine-containing dentifrice before and after grapefruit juice challenge on dentin disc surface with a dab-on application\cite{22} method using circular and horizontal overlapping strokes for 1 min simulating the popular general brushing.\cite{25} Following the treatment with respective agents, a 30-s to 10-min grapefruit juice acid challenge was carried out. Strontium acetate dentifrice observed to deliver statistically significantly greater levels of dentin tubule occlusion after brushing and dietary acid challenge when compared to the arginine dentifrice suggesting that the arginine-containing dentifrice may be more susceptible to acid-mediated dissolution than the strontium-based dentifrice.

A 0.3% citric acid solution with NaOH with a pH of 3.2 was used in this study. Conboy and Cox\cite{23} tested the effects of three dietary acids, phosphoric acid (0.055%) citric acid (0.1M) and oxalic acid (0.05%) which are common component of fruit juices and soft drinks and found that citric acid had maximum demineralizing effect with a pH range 3–4. Further, a series of studies indicate that the erosive potential of an acidic drink is not entirely dependent on its pH, but is strongly affected by its buffering capacity, suggesting that greater the buffering capacity of the drink, longer it will take for the saliva to neutralize the acid.\cite{24}

CLSM is a technique for obtaining high-resolution optical images and has the ability to acquire in-focus images from selected depths through a process known as optical sectioning.\cite{25,26} A nonfluorescent, reflectance mode was used for calculating the percentage of occluded tubules in this study.\cite{21} A new grading system was devised to quantify and compare the percentages of occluded dentinal tubules by the two agents used in our study. Higher grade signified better tubular occlusion by the desensitizing agent. In addition, the range of tubules occluded could be categorized with ease.

The results of this research demonstrated that arginine-containing dentifrice showed higher percentage of tubule occlusion without citric acid challenge as compared to NovaMin, but, NovaMin containing dentifrice resisted the citric acid attack showing an insignificant reduction in the percentage of tubule occlusion.

However, this study should be interpreted with caution before extrapolating them in dental practice, considering the nature and limitations of the in vitro experimentation. 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. We devised a novel grading system, however, future directions can be employed in designing of more sensitive and specific grading systems. 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

This in vitro study, comparing the effects of two relatively newer desensitizing agents namely NovaMin and arginine with respect to tubule occlusion, provides a promising insight in our search for an effective, clinically applicable desensitizing agent. Arginine containing dentifrice showed a higher grade of tubule occlusion, though NovaMin resisted the acidic challenge better. Therefore, based on the findings of our study, future directions in formulating a new combination dentifrice may be employed.

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Conflicts of interest

There are no conflicts of interest.

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