Original Article

A scanning electron microscope analysis on the effect of bleaching gel in combination with fluoro calcium phosphosilicate-based dentifrice on whitening of teeth and dentinal tubule occlusion – An *in vitro* study

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Abstract

Aim: The aim of this study is to investigate the use of 15% carbamide peroxide in combination with fluoro calcium phosphosilicate desensitizing dentifrice during bleaching to evaluate postoperative dentinal tubule occlusion.

Materials and Methods: Premolars which were extracted and bleached using 15% carbamide peroxide with fluoro calcium phosphosilicate dentifrice were used in this investigation, and the shade change was assessed. They were subsequently divided into sections and examined with scanning electron microscope. The quantity of open tubules, fully obstructed as well as moderately obstructed tubules, was recorded for each specimen. The statistical analysis of shade changes was conducted using the paired *t*-test. The tubule numbers were statistically evaluated after being assigned a mean and standard deviation.

Results: The mean values between test and control groups in completely blocked tubules are 81.370 ± 1.989 and 42.586 ± 11.316, respectively. In partially blocked tubules, the mean values were found to be 13.219 ± 1.747 and 32.264 ± 11.554, respectively. In open tubules, the mean values are 5.398 ± 1.260 and 25.149 ± 8.781. The mean values of shade comparison for test group and control group are 1.95 ± 2.78 and 3.40 ± 2.83, respectively.

Conclusions: According to the findings of the study, dentinal tubules were blocked when fluoro calcium phosphosilicate dentifrice was administered with 15% carbamide peroxide, but the bleaching process was unaffected. The potential clinical advantage of using a single paste system for both bleaching and desensitizing was also emphasized.

Keywords: Dentifrice; dentinal tubule; fluoro calcium phosphosilicate

INTRODUCTION

In recent times, a growing quantity of dental care products concentrates on tooth whitening. This is because many people prefer white-shaded teeth and a bright smile because they assume that it would enhance their quality of life. Teeth can get darker as a result of lifestyle choices including smoking, drinking red wine, or drinking black tea. In addition, the age of the teeth also has an impact on tooth color in general.[1]

One of the frequent treatment modalities utilized for teeth whitening is bleaching. Bleaching system is the less invasive

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treatment for discolored teeth due to its popularity. However, tooth-whitening techniques may irritate the gingiva and increase sensitivity.[2]

Bleaching agents can change the enamel's surface in ways that make the dentin more vulnerable to postoperative dental sensitivity, including depressions on enamel, porosity, and irregularities on the surface. In addition, severe tooth sensitivity following bleaching can also be brought in cases of defective and incorrect restorations, gingival recession, and cementoenamel junction defects.[3]

Patients who underwent bleaching therapy with a 10% carbamide peroxide solution experienced a rise in tooth sensitivity ranging from 15% to 65%. Most of the peroxide concentrations and delivery methods have reported this effect.[4]

Despite being the most popular and very effective approach used by clinicians to whiten teeth, at-home bleaching does not completely eliminate hypersensitivity. Dental sensitivity caused by bleaching is a common discomfort that can occur during and following therapy. However, the exact cause of this sensitivity remains unclear and cannot be readily determined. The hydrodynamic theory is commonly employed to explain this condition.[5]

Dentin hypersensitivity (DH) is a chronic condition that causes sharp and temporary pain in noncarious teeth. It can be induced by various stimuli, including temperature changes (hot or cold), chemical changes (acidic, sweet, or salty), and mechanical changes caused by external forces that expose the dentinal tubules.[6] The presence or absence of gingival recession in DH can or cannot associate with erosion and abrasion conditions. However, they are the key etiological factors.[7]

After analyzing different diagnostic and measurement methods and considering different age groups mentioned in the published literature, it was found that the prevalence of DH ranged from approximately 5% to 62%. The most accurate average value globally is 34%.[8]

DH is still a widespread condition that can be treated using two different methods: the utilization of bioactive formulations such as resin-based bonding agents, glutaraldehyde, amorphous calcium phosphate-based tooth mousse, restorative materials, and lasers widely used in dental clinics.[9]

There are several desensitizing toothpastes available in the markets which are composed of bioactive glass (BAG), fluorides, potassium, strontium, and arginine.[10]

One of the most popular BAGs used in the treatment of DH is fluoro calcium phosphosilicate-based dentifrices, which is a category of biomaterials known as "bioactive glasses." Generally, they are included in a variety of dentifrices as finer particles to effectively distribute phosphorus and calcium to the surface of the tooth. When BAG particles are administered as dentifrices, they attach to the dentin and form a layer of hydroxycarbonate apatite that blocks the tubules and relieves pain for extended periods of time.[11]

Although all bioactive treatments significantly reduce DH, currently there are no clarity and availability of limited research data on any product's or bioactive agent's qualitative efficacy. Hence, this *in vitro* study was done to assess the efficiency of a novel desensitizing agent containing fluoro calcium phosphosilicate dentifrice in reducing DH after bleaching procedure in combination with bleaching agent by blocking of dentinal tubules.

The aim of this research was to study the effect of fluoro calcium phosphosilicate desensitizing dentifrice combined with 15% carbamide peroxide on teeth bleaching and occlusion of dentinal tubule.

MATERIALS AND METHODS

The study was conducted in accordance with the Helsinki Declaration of 1975, which was amended in 2000. Since this was an *in vitro* study that did not involve any patients, there was no need for written informed consent from patients.

Inclusion and exclusion criteria

A total of 30 noncarious premolars were obtained from patients aged 18–30 years for this study. For the investigation, teeth with dental caries, cervical abnormalities, cracks, and fractures were eliminated. To prevent the consequences of carbamide peroxide on the surfaces of the root, ultrasonic debridement followed by the application of nail varnish was done. The process of matching shades was achieved by utilizing a Vitapan Classical shade guide manufactured by Vita Zahnfabrik in Germany.

The materials used in the study were as follows:

a. The bleaching agent gel used in this study comprised 15% carbamide peroxide (Opalescence Tooth whitening systems, Ultradent Products Inc., USA)
b. A novel dentifrice used is fluoro calcium phosphosilicate-based desensitizing toothpaste (Elsenz, Group Pharmaceuticals, Malur, Karnataka).

The sample size was estimated using the software GPower version. 3.1.9.2 (GPower, Heinrich Heine University, Dusseldorf, Germany). Based on the mean and standard deviation obtained from the study, the effect size calculated was 0.916, with 0.05 probability of alpha error and "power

of the study" 80%. The "sample size" calculated per group was 20, and the total sample size was 40.

The total samples were segregated into two groups based on the use of dentifrice in combination with bleaching gel. In Group 1 (test) with sample size ($n = 20$) combination of fluoro calcium phosphosilicate dentifrice and 15% carbamide peroxide was used. In Group 2 (control) with sample size ($n = 20$), the samples were bleached with 15% carbamide peroxide without fluoro calcium phosphosilicate dentifrice, and the tubule occlusion was observed. The tea solution was prepared for staining by soaking 20 g of tea (black tea) in 150 ml of hot water for 24 h.

Bleaching procedure

All of the teeth were bleached using a 1:1 mixture of desensitizing toothpaste containing fluoro calcium phosphosilicate and 15% carbamide peroxide. With this novel mixture, bleaching was accomplished by placing teeth in a disposable glass for 8 h each day, and the procedure was carried out for 2 weeks. To replenish the bleaching agent, a fresh solution was prepared each day by blending desensitizing toothpaste containing fluoro calcium phosphosilicate and 15% carbamide peroxide in a 1:1 ratio. Following a thorough cleansing with distilled water, the same three examiners took a new shade postbleaching, and thick slices of "1 mm" were cut right above the cementoenamel junction using a diamond disc and a micromotor (NSK products, Tochigi, Japan).

Dentinal tubule evaluation

The occlusion of dentinal tubules was evaluated using a scanning electron microscope (SEM), (JEOL Pvt. Ltd., USA), obtained at ×2000 and 10KV. Tubule counting was performed on all images. Three observers independently analyzed each image and calculated the quantity of fully obstructed tubules, open tubules, and partially obstructed tubules. Tubules that were not entirely visible on the SEM images were excluded from the count. The tubule counts from the three observers for each SEM image were averaged to determine the quantities of open, partially occluded, and total visible (open and partially occluded) tubules.

Shade evaluation

The samples of teeth were maintained in a vacuum-sealed jar between bleaching sessions and were stored in another disposable glass. Three examiners used a Vitapan Classical shade guide (Vita Zahnfabrik, Bad säckingen, Germany) to record the shade in natural sunlight. To assess variations in tooth color, the 16 tabs of the shade guide were organized in ascending order, with the highest value (B1) at the top and the lowest value (C4) at the bottom. The tooth shade alterations were determined by calculating the shift in the number of shade guidance units toward the lighter end of the scale. Three persons conducted shade matching using natural daylight, and the average of their observations was used for the analysis.

Statistical analysis

The analysis was performed using "SPSS Statistical Package for the Social Sciences version 22.0, IBM, Pvt. Ltd., Chicago, USA." "Intergroup comparison" was performed using "unpaired *t*-test," and "intragroup comparison" was performed by "paired *t*-test." For all comparisons, $P < 0.05$ was considered to be "statistically significant."

RESULTS

Dentinal tubule occlusion

Figure 1 demonstrates the SEM image of blocked tubules, and Table 1 depicts the mean percentage of dentinal tubule occlusion evaluated in Group 1 (test). The mean percentage value of completely blocked tubules is 81.37 ± 1.99, and Table 2 depicts the mean percentage of dentinal tubule occlusion evaluated in Group 2 (control), and a mean value of 42.58 ± 11.31 was observed. Figure 2 demonstrates the SEM image of partially blocked tubules, and the mean of partially blocked tubules in Group 1 was found to be 13.22 ± 1.7, and in Group 2, it was found to be 32.26 ± 11.55. Figure 3 demonstrates the SEM image of open tubules, and the mean for the number of open tubules was 5.4 ± 1.2 in Group 1, and in Group 2, it was found to be 25.14 ± 8.78. An intergroup comparison was tabulated using unpaired *t*-test which shows the mean value between test and control groups in completely blocked tubules as 81.370 ± 1.989 and 42.586 ± 11.316 [Table 3]. In partially blocked tubules, the mean value was found to be 13.219 ± 1.747 and 32.264 ± 11.554. In open tubules, the mean value is 5.398 ± 1.260 and 25.149 ± 8.781.

Comparison of shade

In the present study, comparison of the mean shade difference between Group 1 and Group 2 was evaluated.

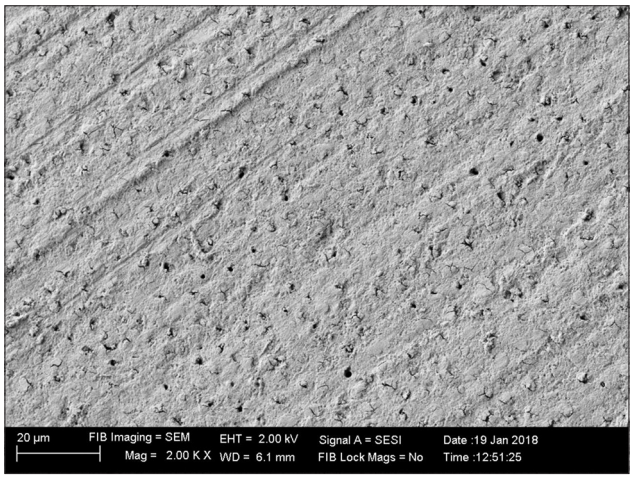

Figure 1: Scanned electron microscope image of blocked tubules

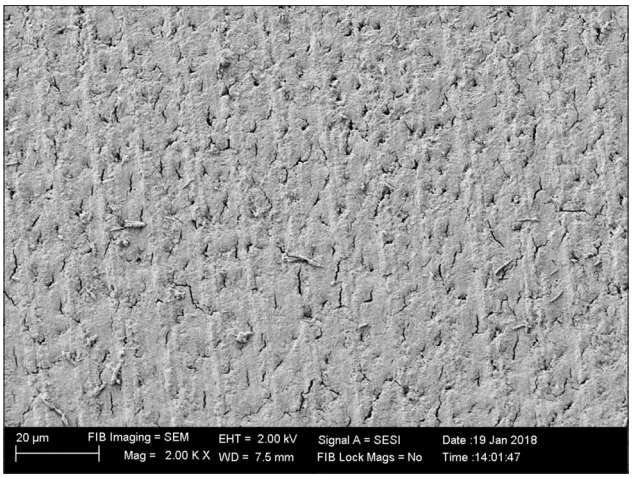

Figure 2: Scanned electron microscope image of partially blocked tubules

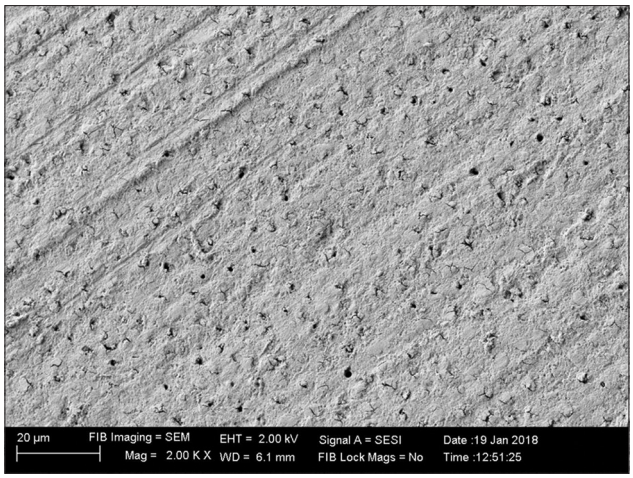

Figure 3: Scanned electron microscope image of opened tubules

Intergroup comparison between Group 1 and Group 2 was performed using unpaired *t*-test with a mean value of 1.95 ± 2.78 for test group and 3.40 ± 2.83 for control group [Table 4].

Table 1: Statistical analysis of mean percentage of dentinal tubules in test group

	Group 1 (test)		
Serial number	Percentage complete blocked	Percentage partially blocked	Percentage open tubules
1	80.55	14.81	4.62
2	83.49	10.67	5.82
3	83	13	4
4	82.07	13.20	4.71
5	82.35	11.76	5.88
6	81.30	14.95	3.73
7	86.31	10.52	3.15
8	83.49	10.67	5.82
9	80	13.33	6.66
10	79.62	14.81	5.55
11	80.41	12.37	7.21
12	82.17	12.87	4.95
13	79.09	13.63	7.27
14	79.61	16.50	3.88
15	78.78	16.16	5.05
16	81.05	12.63	6.31
17	83.96	12.26	3.77
18	79	15	6
19	79.61	13.59	6.79
20	81.55	11.65	6.79
Mean±SD	81.37±1.990	13.22±1.746	5.40±1.26

SD: Standard deviation

DISCUSSION

Patients frequently complain of postoperative tooth discomfort following vital bleaching. Since tooth whitening typically requires several sessions to attain the desired shade, patient may experience unpleasant tooth sensitivity during the initial visit which may be a factor in their decision to discontinue treatment. During essential in-office bleaching procedures, several research investigations have emphasized the significance of using substances that reduce sensitivity in managing tooth sensitivity following the procedure. Postoperative tooth discomfort is more prevalent due to a lack of comprehensive studies, as the concentration of the bleaching agent used in postoperative treatments is considerably higher compared to at-home bleaching therapies.[12]

Tooth wear and gingival recession are among the common etiological factors which lead to DH, which is a frequent condition which significantly lowers the quality of a healthy tooth. Several treatments and materials have been developed for the control of DH. The most conventional method for making an antihypersensitivity dentifrice is to add 5% KNO3 (potassium nitrate) to "sensitive formula" gels. This approach, however, disregards the fact that consistent, long-term use is required to significantly reduce sensitivity.[13] The most effective fluoride formulations for reducing hypersensitivity contain stannous fluoride of 0.4%. Therefore, adding additional fluorides may not be an effective choice due to this drawback.[14]

This method typically results in products that are a little unstable and unresponsive, which can cause severe tooth discoloration. As a result, it is not recommended to employ this component in bleaching gels. Manufacturers often provide formulations including sodium fluoride, but they are inferior, slow-acting alternatives.[15]

Fluoro calcium phosphosilicate, which is a bioglass containing fluoride, has been produced and developed to serve as a dentifrice for desensitization purposes. This formulation distinguishes itself from toothpastes containing bioglass which was used earlier by having a higher concentration of phosphate, as well as the inclusion of calcium fluoride in the glass particles and a tiny average particle size. The process results in the production of fluorapatite, rather than hydroxyapatite, on the teeth.

Table 2: Statistical analysis of mean percentage of dentinal tubules in control group

	Group 2 (control)		
Serial number	Percentage complete blocked	Percentage partially blocked	Percentage open tubules
1	60	20	20
2	50	30	20
3	38.09524	38.09524	23.80952
4	29.41176	58.82353	11.76471
5	50	14.28571	35.71429
6	31.25	31.25	37.5
7	25	37.5	37.5
8	42.10526	31.57895	26.31579
9	55.55556	27.77778	16.66667
10	44.44444	33.33333	22.22222
11	60	20	20
12	50	30	20
13	38.09524	38.09524	23.80952
14	29.41176	58.82353	11.76471
15	50	14.28571	35.71429
16	31.25	31.25	37.5
17	25	37.5	37.5
18	42.10526	31.57895	26.31579
19	55.55556	27.77778	16.66667
20	44.44444	33.33333	22.22222
Mean±SD	42.586±11.316	32.264±11.554	25.149±8.781

SD: Standard deviation

Table 3: Statistical analysis of test and control using unpaired *t*-test

Group	*n*	Mean±SD	*t*	df	*P*
Complete blocked					
Group 1 (test)	20	81.374±1.990	14.744	28	<0.001
Group 2 (control)	10	42.586±11.626			
Partial blocked					
Group 1 (test)	20	13.222±1.746	−7.144	28	<0.001
Group 2 (control)	10	32.264±11.871			
Open tubules					
Group 1 (test)	20	5.402±1.260	−9.769	28	<0.001
Group 2 (control)	10	25.149±9.021			

SD: Standard deviation

Table 4: Intergroup shade evaluation using paired *t*-test

Groups	*n*	Mean±SD	*t*	df	*P*
Group 1 (test)	20	1.9500±2.78104	−1.326	28	0.196
Group 2 (control)	10	3.4000±2.91357			

SD: Standard deviation

Fluorapatite is more resistant to acids produced by microbes, which increases remineralization. This is due to the combination of calcium and phosphate released from the glass particles. In 2016, the fluoro calcium phosphosilicate-based dentifrices were introduced for sale in the United Kingdom through online platforms and in selected pharmacies in Germany and India.[16]

The Food and Drug Administration has given its approval to fluoro calcium sodium phosphosilicate-based products. Numerous investigations have concentrated on the reduction in sensitivity that occurs after open dentinal tubules are blocked.[17] SEMs were used in most of these experiments to show the open tubules before treatment and the blocked tubules after treatment.[18]

According to current research, restricting dentinal tubules (either temporarily or permanently) can relieve hypersensitivity. This aligns with Brannstrom's concept of hydrodynamic fluid movement in the tubules that leads to sensitivity (Brannstrom, 1963). Research using toothpastes containing calcium sodium phosphosilicate consistently showed a significant reduction in tooth sensitivity.

The intergroup comparison tabulated using unpaired *t*-test in Table 3 shows the mean value between test and control groups in completely blocked tubules as 81.370 ± 1.989 and 42.586 ± 11.316. In partially blocked tubules, the mean value was found to be 13.219 ± 1.747 and 32.264 ± 11.554. In open tubules, the mean value is 5.398 ± 1.260 and 25.149 ± 8.781. There was a statistical significant difference in the percentage of completely blocked, partially blocked, and open dentinal tubules between the study and control group that is $P < 0.001$. $P = 0.111$ was observed in comparison of the mean shade difference between Group 1 and Group 2 evaluated in Table 4 which depicts the intergroup comparison between Group 1 and Group 2 using unpaired *t*-test with mean value of 1.95 ± 2.78 for test group and 3.40 ± 2.83 for control group.

The results of this study align with studies conducted by "Kakodkar *et al*.," in which dentinal tubule occlusion was evaluated using bleaching gel enriched with calcium sodium phosphosilicate-based dentifrice on whitening of teeth. They believed that adding dentifrice to 15% carbamide peroxide occluded the dentinal tubules and had no effect on the bleaching process.[19]

Previous *in vivo* research studies conducted by Rajesh *et al*. on evaluation of calcium sodium phosphosilicate-based dentifrice were found successful in reduction of DH.[20] Similar research by "Milleman *et al*." observed no significant harmful effects of calcium sodium phosphosilicate-based dentifrice when compared with a prophylaxis paste.[21]

Ashwini *et al*. conducted a randomized controlled clinical trial on 5% fluoro calcium phosphosilicate dentifrice's desensitizing effectiveness in comparison with 5% calcium sodium phosphosilicate. In the fluoro calcium phosphosilicate group, there was a clinically significant decrease in the sensitivity response.[22] The study conducted by Aggarwal *et al*. demonstrated that compared to other dentifrices, fluoro calcium phosphosilicate BAG-containing desensitizing dentifrices have an early onset of action in alleviating hypersensitivity, which may lead to a superior treatment response for the treatment of DH.[23] Penha *et al*., in their study, also found that BAGs with Nd:YAG laser on

the dentin surface may be a promising alternative for the reduction of dentin permeability.[24]

Limitations of the study

Considering the methodology of this *in vitro* research, it is acknowledged that there were several limitations present. It was not determined how deeply the desensitizing agents penetrated the dentinal tubules. Greater depth of penetration into dentinal tubules could lead to prolonged relief from dentinal hypersensitivity. With the use of carbamide peroxide, the ability of the occluded tubules to resist the acidic reaction was not evaluated. Although efforts were made to replicate clinical conditions, the use of distilled water and tea solution was used to store and stain the samples for a particular duration of time instead of artificial saliva, phosphate-buffered saline, and dyes.

CONCLUSIONS

Considering all the limitations of the study according to this *in vitro* research, it can be emphasized that dentinal tubules were blocked when fluoro calcium phosphosilicate dentifrice was administered in combination with 15% carbamide peroxide; however, the bleaching process was unaffected. The utilization of a single-paste technique for both desensitization and bleaching can help in achieving both therapeutic benefits in clinical scenario. Fluoro calcium phosphosilicate-based dentifrices are promising novel biomaterials which have the potential to be used as adjuncts in treatment modalities; however, further *in vitro* and *in vivo* research is required for further development of such products.

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Conflicts of interest

There are no conflicts of interest.

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