ORIGINAL ARTICLE

An In Vitro Evaluation of the Remineralizing Efficacy of Two concentrations of Silica Doped Nanohydroxyapatite on Bleached Enamel

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ABSTRACT

Introduction: Vital bleaching of teeth is associated with mineral loss and surface roughness leading to hypersensitivity. Aesthetic restorations are recommended after one week. Search is on for a suitable remineralizing material, which helps in instant adhesive bonding. Hence objective of the study is to evaluate the remineralizing efficacy of two concentrations of Silica doped Nanohydroxyapatite on bleached enamel. **Methods:** Enamel surfaces of 30 extracted human central incisors were divided into Part A: Unbleached enamel, Part B: Bleached enamel, Part C: Remineralized enamel. The samples were randomly divided into, Group 1: MI Paste Plus (Recaldent, USA), Group 2 and 3 for application of Dentin bonding agents (Tetric- n-bond, Ivoclar, Vivadent) mixed 0.2% and 0.8% Silica doped Nanohydroxyapatite (Sigma Aldrich, Bangalore, India). Post bleaching remineralizing agents were applied on part C. Surface roughness was evaluated with contact stylus profilometer and mineral content was evaluated with Energy dispersive X-Ray spectroscopy for three parts. Data were analysed using ANOVA and Post Hoc Tukey test with $p \leq 0.05$. **Results:** Surface roughness values (Ra) were increased, and mineral loss (Ca:P) was observed after bleaching. After application of remineralizing agents, surface roughness was decreased with no significant value ($p > 0.05$) and a significant increase in mineral content of all three groups with a $p < 0.05$ was observed. **Conclusion:** Application of dentin bonding agent mixed with Silica doped Nanohydroxyapatite decreased surface roughness and improved remineralization of bleached enamel.
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Keywords: MI Paste Plus, Remineralization, Silica doped nanohydroxyapatite, Surface roughness, Tooth Bleaching

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INTRODUCTION

Effective communication depends on the confidence level of an individual. Good appearance with an aesthetic smile gives a boost to the confidence level. In this regard, nurturing the natural teeth and treatment, if required for any discoloration of teeth, has gained popularity (1). Treating discoloured tooth has been introduced in the 1880s. Its research has become more popular by the works of Hayman and Haywood in the year 1989. Millions of people are being benefited by the treatment of discolorations since the invention of bleaching products and protocols (2).

Vital tooth bleaching involves bleaching the surface of the enamel with hydrogen peroxide or carbamide peroxide (CP) at different concentrations (3). The commonly used concentrations of the carbamide peroxide bleaching gels are 15 to 22 %, and the bleaching protocol is for two weeks. However, these products led to few undesirable adverse effects. The mineral content is decreased because of slight acidic PH of bleaching agents leading to surface roughness. The low molecular weight oxidative free radicals produced by dissociation of bleaching agents produce porosities in enamel surface and permit the bleaching solution diffuse in the dentin leading to hypersensitivity (4-8).

To overcome these adverse effects, research is going on for the reversal of these adverse effects. MI Paste Plus is a potential remineralizing agent, used to treat demineralized surfaces for the promotion of mineralization with application for two weeks. The amorphous calcium phosphate (ACP) systems stabilized by casein phosphopeptide (CPP), provides a higher reservoir of bioavailable calcium and phosphate ion. This aids in decreasing the surface roughness and increase in microhardness by deposition of the mineral content on the bleached enamel. There is evidence that MI Paste

Plus showed a better result in remineralization than ACP gels (9). Immediate composite restorations cannot be done after applying MI Paste Plus as its application requires two weeks according to the manufacturer instructions for better surface remineralisation. Hence search is on for a suitable remineralizing material, which helps in instant bonding.

Hydroxyapatite is the major component of the enamel and bone. Many researchers investigated hydroxyapatite biomineralization in structures like bone and enamel. Element doping of hydroxyapatite by silica plays a more prominent role in biomineralization (10). In this direction, thought was directed towards novel material, Silica doped nano-hydroxyapatite (Si-DNH), a remineralizing agent that was used to improve the mineral deposition on the surface of the bone. By mixing it with a dentine bonding agent (DBA), it becomes part of the adhesive protocol for immediate composite restorations and might help in remineralization.

This study was designed to evaluate the effect of Si-DNH on remineralization of bleached enamel with the null hypotheses that there will not be any change in surface roughness and mineral content after application of Si-DNH.

MATERIALS AND METHODS

Sample size calculation

It was performed for surface roughness and mineral content as the primary outcome using G Power 3.1.9.2 considering 95% confidence level with 80% power with effect size of 0.68 based on the results of pilot study. The estimated desired sample size was 24, but it was made to 30 to include 10 samples in each group.

Samples used for study

Extracted human maxillary central incisors satisfying the inclusion criteria, were collected with the approval from ethical committee. (VDC/IEC/2017/32). Inclusion criteria were anatomically and morphologically well-defined teeth without caries with intact tooth structure. Exclusion criteria were teeth with heavy restorations, fractured crowns, hypoplastic lesions, teeth with fluorosis, non-carious enamel lesions.

Samples were examined under the dental operating microscope (Carl Zeiss Surgical GmbH, Oberkochen, Germany) at 1 X equivalent to 8.5 times magnification to evaluate for enamel cracks and surface debris. 30 samples were stored in artificial saliva during the entire experimental procedure in airtight plastic containers.

The surface characters and mineral contents will be unique for each tooth. Every tooth is unique in its surface characteristics and mineral content. This model can create a reliable baseline with similar morphology and enamel substrate. The confounding factors associated with enamel heterogenicity like age, mineralization, and hardness. (11) So, to reduce the confounding factors, all the experimental protocols and comparative evaluations were performed on the same tooth.

The enamel surface of each tooth was divided into three parts mesiodistally as Part A, B, C, respectively. Part A was covered with surgical micropore tape to prevent the bleaching material from entering on the enamel surface, and it acts as a standard sound enamel. On the enamel surfaces of Part B and Part C, bleaching procedure was performed. At the end of the bleaching session remineralizing agents were applied to Part C of their respective groups. Care was taken to prevent spread of remineralizing agent on Part B under magnification. Teeth were stored in artificial saliva after completion of every bleaching cycle.

Division of Groups

30 samples were divided into three groups with ten in each group using computer- generated randomization (Random.org)
Group 1: Remineralization with MI Paste Plus
Group 2: Remineralization with dentin bonding agent containing 0.2 wt. % Si-DNH
Group 3: Remineralization with dentin bonding agent containing 0.8wt. % Si-DNH

Bleaching Regimen

22% Carbamide peroxide gel (Zoom Nitewhite, Phillips) was applied to the part B and C of labial surfaces of all the specimens for eight hours per day for two weeks. After each bleaching session, the samples were rinsed with distilled water thoroughly to remove any remnant bleaching gel on the surface using a 5ml disposable syringe and stored in artificial saliva.

Group 1: Part C was treated with MI Paste Plus. A small amount of MI Paste Plus was applied on the enamel surface for 15 minutes. After that, samples were rinsed off with distilled water with syringe and stored in artificial saliva until next bleaching cycle.

Preparation of Remineralizing Solution

Total etch dentin adhesive (Tetric- n-bond, Ivoclar, Vivadent) was chosen for the study. The weight of the Si-DNH (Sigma Aldrich, Bangalore, India) was measured using a microbalance and the volume of the solution using 1 ml glass syringes. 0.2 wt.% Si-DNH containing dentin bonding agent was prepared by mixing 2 mg of Si-DNH powder into 1 g of the dentine bonding agent (Tetric- n-bond, Ivoclar, Vivadent). 0.8 wt.% Si-DNH containing dentine bonding agent was prepared by mixing 8 mg of Si-DNH powder into1 g of the dentine bonding agent.

The obtained solution was homogenized by ultrasonication using a probe Sonicator apparatus (PCI Analytics, India) at 20% power and sonication time of

20 seconds. Two cycles were performed with a gap of 20 seconds. Dentine bonding agent was immersed in an ice-water bath during sonication to prevent the loss of volatile solvent (ethanol) from the bonding agent due to heat generated during sonication (12). A freshly prepared dentine bonding agent was used as a remineralizing solution on bleached enamel.

Group 2: At the end of the bleaching session, the bleaching agent was rinsed off from the surface using distilled water. Enamel surface in Part C was etched with 37% orthophosphoric acid (N-Etch, Ivoclar vivadent, USA) for 15 seconds and rinsed off thoroughly using distilled water with syringe and blot dried.

Freshly prepared 0.2 wt.% Si-DNH remineralizing solution was applied to Part C using the tip of the sterile probe. It was cured with a blue phase LED curing light (3M India Ltd) for 20 seconds with a light intensity of 1100 Mw.

Group 3: Freshly prepared 0.8 wt. % Si-DNH remineralizing solution was applied to Part C using the tip of the sterile probe.

Evaluation of surface roughness using Profilometer

All the specimens were tested for surface roughness using a contact stylus profilometer at three different thirds of the teeth (13). Mitutoyo Surftest profilometer was used as per the manual. The scanning arm with measuring force of 4 mN moved with the speed of 0.25mm/s. The arithmetic average height of surface irregularity from the mean line was considered as Ra parameter in µm. The mean of five readings was considered as Ra of each sample. Before the experiment the pin was calibrated. In order to exclude possible errors, measurement of surface roughness was done by only one examiner.

Evaluation of mineral content

All samples were decoronated using a double-faced diamond disc attached to a laboratory micromotor, tested for mineral content at three different thirds of the teeth using Energy dispersive X-Ray spectroscopy (14,15).

Statistical Analysis

All the obtained values were statistically analysed using ANOVA test and confirmed using Post Hoc Tukey's test with p value < 0.05. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. was used for statistical analysis.

RESULTS

Comparison of mean, standard deviations, and significance values at different portions of teeth using different remineralizing agents for surface roughness are summarized in Table I and mineral content are summarized in Table II. Surface roughness increased in all groups from Part A to Part B and decreased in Part C with no significant value in all groups, whereas mineral content decreased from Part A to Part B and increased in Part C with significant value of P=0.001 in all groups. Comparison of surface roughness in different groups at different parts on tooth surface are summarized in Table III and mineral content are summarized in Table IV. Surface roughness has decreased in all three groups of remineralizing agents with no significant difference. Mineral content has increased in all three groups of remineralizing agents with significant value of P=0.001 Since significant difference was observed in mineral content values Post Hoc Tukey analysis was done and summarized in Table V and Table VI. A significant difference was observed between MI Paste Plus and 0.2 wt.% Si-DNH with P=0.001. A significant difference was also observed between MI Paste Plus and 0.8 wt.% Si-DNH with P=0.007. No significant difference was observed between 0.2 wt.% Si-DNH and 0.8 wt.% Si-DNH. Table VI demonstrates a significant difference from Part A to Part B, and Part B to Part C. No significant difference was observed between Part A to Part C.

Table I: Comparison of surface roughness (Ra) at different portions of teeth using MI Paste Plus, 0.2wt.% Si-DNH, 0.8wt.% Si-DNH

Groups	Parts on tooth surface	Mean	Standard deviation	F value	p value
MI Paste plus	**Part A**	0.2158	0.03942		
	Part B	0.2542	0.03770	2.424	0.108
	Part C	0.2363	0.03993		
0.2 wt.% Si-DNH	**Part A**	0.2133	0.05436		
	Part B	0.2607	0.05748	1.720	0.198
	Part C	0.2493	0.06644		
0.8 wt. % Si-DNH	**Part A**	0.2239	0.06743		
	Part B	0.2722	0.06542	1.424	0.258
	Part C	0.2484	0.05883		

* Significant (p ≤ 0.05) non-significant (p > 0.05)

Table II: Comparison of mineral content (Ca:P) at different portions of teeth using MI Paste Plus, 0.2wt.% Si-DNH, 0.8wt.% Si-DNH

Groups	Parts on tooth surface	Mean	Standard deviation	F value	p value
MI Paste plus	**Part A**	2.3070	.028304		
	Part B	1.5500	0.55422	10.287	0 .000*
	Part C	1.9880	0.18426		
0.2 wt.% Si-DNH	**Part A**	2.6220	0.26415		
	Part B	1.6800	0.29687	36.596	0 .000*
	Part C	2.5170	0.24608		
0.8 wt. % Si-DNH	**Part A**	2.3900	0.20067		
	Part B	1.5000	0.28028	38.143	0 .000*
	Part C	2.3450	0.28084		

* Significant (p ≤ 0.05) non-significant (p > 0.05)

DISCUSSION

Tooth enamel is the highly mineralized tissue of the

Table III: Comparison of surface roughness (Ra) in different groups at Part A, Part B and Part C

Parts on tooth surface	Groups	Mean	Standard deviation	F value	p value
Part A	**MI Paste plus**	0.2158	0.03942	0.102	0.904
	0.2 wt.% Si-DNH	0.2239	0.06743		
	0.8 wt.% Si-DNH	0.2133	0.05436		
Part B	**MI Paste plus**	0.2542	0.03770	0.277	0.760

* Significant (p ≤ 0.05) non-significant (p > 0.05)

Table IV: Comparison of mineral content in different groups at Part A, Part B and Part C

Parts on tooth surface	Groups	Mean	Standard deviation	F value	p value
Part A	**MI Paste plus**	2.3070	0.28304	4.206	0.026*
	0.2 wt.% Si-DNH	2.6220	0.26415		
	0.8 wt.% Si-DNH	2.3900	0.20067		
Part B	**MI Paste plus**	1.5500	0.55422	0.547	0.585
	0.2 wt.% Si-DNH	1.6800	0.29687		
	0.8 wt.% Si-DNH	1.5000	0.28028		
Part C	**MI Paste plus**	1.9880	0.18426	12.599	0.000*
	0.2 wt.% Si-DNH	2.5170	0.24608		
	0.8 wt.% Si-DNH	2.3450	0.28084		

* Significant (p ≤ 0.05) non-significant (p > 0.05)

human body, which is composed of 90 % mineral salts and 10% of water and organic substances. Small amounts of fluoride, calcium carbonate, sodium, potassium are also present. This inorganic material is mainly composed of calcium and phosphate bound to hydroxyapatite (3). The surface morphology and mineral content is unique to the individual tooth. The intra-tooth model used in the present study was an effort to provide same baseline enamel substrate so that all procedures and evaluations were made on the same sample increasing the internal validity (11).

The most used dental bleaching products are hydrogen peroxide and carbamide peroxide (3) with concentrations ranging from 10 % to 40% (4). They enhance the appearance of the discoloured tooth structure employing some redox reactions. However, this is associated with change in surface topography and mineral content.

These surface changes can be evaluated qualitatively as well as quantitatively. The qualitative evaluation includes morphological analysis by SEM (6). However, quantitative measurements like surface roughness (7,8,13,16-18) with profilometer (7,13,19), microhardness (19), Atomic force microscopy, Raman spectroscopy, and EDX can give substantial information regarding the changes (14,15). The adverse effects

Table V: Post Hoc Analysis of mineral content between different remineralizing agents.

Parts	Groups	Compared to	Mean Difference	Standard error	Sig.	Lower bound	Upper bound
Part A	MI Paste plus	0.2 wt.% Si-DNH	-0.31500*	0.11259	0.025*	-0.5942*	-0.0358
	MI Paste plus	0.8 wt.% Si-DNH	-.008300	0.11259	0.744	-0.3622	0.1962
	0.2 wt.% Si-DNH	0.8 wt.% Si-DNH	0.23200	0.11259	0.117	-0.0472	0.5112
Part B	MI Paste plus	0.2 wt.% Si-DNH	-0.13000	0.17773	0.747	-0.5707	0.3107
	MI Paste plus	0.8 wt.% Si-DNH	0.05000	0.17773	0.957	-0.3907	0.4907
	0.2 wt.% Si-DNH	0.8 wt.% Si-DNH	0.18000	0.17773	0.575	-0.2607	0.6207
Part C	MI Paste plus	0.2 wt.% Si-DNH	-0.52900*	0.10751	0.000*	-0.7956*	-0.2624
	MI Paste plus	0.8 wt.% Si-DNH	-0.35700*	0.10751	0.007*	-0.6236*	-0.0904
	0.2 wt.% Si-DNH	0.8 wt.% Si-DNH	0.17200	0.10751	0.263	-0.0946	0.4386

* Significant (p ≤ 0.05) non-significant (p > 0.05)

Table VI: Post Hoc Analysis of mineral content between different parts on tooth surface

Groups	Parts	Compared to	Mean Difference	Standard error	Sig.	Lower bound	Upper bound
MI Paste plus	Part A	Part B	0.75700*	0.16757	0.000*	0.3415*	1.1725
	Part B	Part C	-0.43800*	0.16757	0.037*	-0.8535*	-0.0225
	Part A	Part C	0.31900	0.16757	0.157	-0.0965	0.7345
0.2 wt.% Si-DNH	Part A	Part B	0.94200*	0.12068	0.000*	0.6428*	1.2412
	Part B	Part C	-0.83700*	0.12068	0.000*	-1.1362*	-0.5378
	Part A	Part C	0.10500	0.12068	0.663	-0.1942	0.4042
0.8 wt.% Si-DNH	Part A	Part B	0.89000*	0.11480	0.000*	0.6054*	1.1746
	Part B	Part C	-0.84500*	0.11480	0.000*	-1.1296*	-0.5604
	Part A	Part C	0.04500	0.11480	0.919	-0.2396	0.3296

* Significant (p ≤ 0.05) non-significant (p > 0.05)

caused by the bleaching may cause hypersensitivity and might lead to pulpal inflammation. To prevent this, the best approach would be to remineralize the bleached surface. At times, aesthetic restorative therapy like veneers might be indicated after bleaching. Literature states that evaluation of the mineral values was done at the end of the bleaching cycles or remineralization regimen, generally at 7 days or 14 days. Hence, it may take a period of one or two weeks for the remineralization of the enamel with MI Paste Plus This would be a drawback for this paste to proceed with the immediate composite restorations.

The bleached enamel, immediately after the procedure if restored with composite resin, results in decreased shear bond strength. Maryam, in her study, states that it takes two weeks after the bleaching procedure or after the application of the desensitizer, for regaining the required bond strength (15). For immediate restorations, to enhance the remineralization and adhesive bonding, a novel remineralizing agent Si-DNH with dentine bonding agent has been prepared and evaluated.

In all groups the mineral content decreased after bleaching significantly with p value 0.001. After application of remineralizing agents, a significant increase in the mineral content was observed with a p value of 0.001. Samples with application of 0.2 wt.% and 0.8 wt.% Si-DNH mixed with dentine bonding agent showed significant increase in mineral content in comparison with application of MI Paste Plus as explained in Table V.

Increased surface roughness and decreased mineral content can be explained as follows. Carbamide peroxides upon dissolving in water or saliva dissociates into hydrogen peroxide and urea. Hydrogen peroxide is a potent oxidizing agent. It diffuses through the enamel and the necessary process involves oxidation and reduction reactions, converting organic pigments into the water, oxygen, and free radicals. These free radicals oxidize the pigmented molecules into smaller and lighter molecules (5). The generated free radicals have less molecular weight and can denature proteins. This process leads to creating porosities on the surface of the bleached enamel, which may lead to dentinal hypersensitivity (5). The process of action of CP on the surface of enamel leads to lowering the pH resulting in the surface roughness and mineral loss (4-8).

The surface roughness reduced as there was increase in mineral content after application of remineralizing agents. The results of this study are in accordance with other literature. MI Paste Plus can localize ACP at the tooth structure, increasing the level of calcium phosphate and plaque, and hence, it may act as a calcium phosphate reservoir, buffering the free calcium and phosphate ion activities. This state of supersaturation for tooth enamel decreases enamel demineralization and enhances enamel remineralization. The CPP molecules contain a cluster of phosphoseryl residues that markedly increase the solubility of calcium phosphate by stabilizing amorphous calcium phosphate under neutral and alkaline conditions. Use of fluoride, which is present in MI Paste Plus, helps in maintaining a state of supersaturation by suppressing demineralization (20-23). Application of MI Paste Plus requires about two weeks for complete remineralization, is a drawback for immediate adhesive restorations.

This deficiency can be compensated by the novel remineralizing agent Si-DNH with DBA. It is one-time application and as it is readily available in the bonding agent, becomes a part of dentin bonding protocol for composite restorations. Despite applying once on the tooth surface, 0.2 and 0.8 wt.% Si-DNH have shown better results over MI Paste Plus.

This could be attributed to the presence of nano-sized Si-DNH and its efficient biomineralization. The use of element doped hydroxyapatite is a new technique used to bring special biological functionalities of HA. According to Zhi-Ye et al. (10), there are three types of doping for HA: doping at $Ca2+$ site, doping at $PO43-$, and doping at channel-ion ($OH-$).

As early as the 1970s, the important role of silica in bone mineralization was observed. Calcium and silica are important contents in the progress of calcification. Some studies found Silica increases the extracellular matrix of connective tissue, polysaccharide content of the matrix, and hydroxyproline. Si-DNH demonstrated good osteogenic activity. Si-DNH was thought to produce better remineralization than the previously used remineralizing materials. Researchers have observed that silica doped hydroxyapatite (Si-HA) could have improved bioactive behaviour and can be one of the potential methods for improving the bioactivity of hydroxyapatite (10).

In Vivo studies have stated that 0.8 wt.% Si-HA exhibited the best effect on promoting bone ingrowth and in bone defect repair (10,24). This material, as it is doped to hydroxyapatite, is thought to produce mineralization in the demineralized surfaces. Two different concentrations, 0.2 wt.% and 0.8 wt.% concentrations of Si-DNH, were tested in the study to observe the change in the mineral deposition. Si-DNH provides a site for calcium adsorption. It helps in the better formation of an amorphous apatite layer on the surface of the tooth. Si-HA was proved as a better bioavailability of calcium and proved in the proliferation of osteoblasts and fibroblasts and helped in better bone mineralization (24).

Si-HA up-regulates the osteogenic related gene expressions along with alkaline phosphatase, bone morphogenic protein II and type I collagen. This biomineralization was observed in osseointegration of Si-HA treated implants (25,26). In the present study, improved biomineralization was observed with application of Si-DNH combined with DBA. As far as the authors knowledge, this is the first study which has evaluated efficacy of Si-DNH. The null hypothesis was rejected as there was change in the surface roughness and mineral content values after application of DBA mixed with Si-DNH.

The limitations of the study are the complexities of the oral cavity, like mechanical and pH dynamics were not simulated and future studies must simulate them to understand their effect of on the remineralization potential.

CONCLUSION

Surface roughness values increased because of bleaching. Application of remineralizing agents decreased the surface roughness. Bleaching resulted in decreased mineral content values. Application of 0.2 wt.% and 0.8 wt.% Si- DNH with DBA remineralizing agents increased the mineral content significantly. MI Paste Plus requires multiple applications after every bleaching cycle and requires some time for remineralization. The clinical advantage of Si-DNH over MI Paste Plus is that, as it is a component of adhesive resin, it becomes a part of bonding protocol, and immediate composite restoration can be thought of.

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