Original Article

Comparative analysis of remineralizing efficacy of strontium-doped bioactive glass, BioMin, and NovaMin containing dentifrice on artificial white spot lesions after chlorhexidine pretreatment - An *in vitro* study

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Abstract

Aim: This study aimed to compare the remineralizing efficacy of strontium-doped bioactive glass, BioMin, and NovaMin containing dentifrices on artificial white spot lesions (WSLs) after chlorhexidine (CHX) pretreatment using energy-dispersive X-ray analysis (EDAX).

Materials and Methodology: Twenty-four samples of maxillary first premolars extracted for orthodontic purposes were selected. Artificial WSLs were produced by immersing the samples in a prepared demineralizing solution for 3 days. All the samples were pretreated with CHX and divided into four groups and six samples each. Group A treated with strontium-doped bioactive glass, Group B with BioMin, Group C with NovaMin, and Group D with artificial saliva as the control group for 14 days. pH-cycling model was used to simulate the oral cavity changes. EDAX was used to record the values at baseline, demineralization, and after remineralization.

Statistical Analysis: Data were analyzed using one-way ANOVA (*post hoc*), followed by unpaired *t*-test and Scheffe tests with Statistical Package for the Social Sciences version 16.

Results: Strontium-doped bioactive glass exhibited a greater mineral regain compared to BioMin, NovaMin, and control groups.

Conclusion: Strontium-doped bioactive glass has enhanced mineral deposition on carious teeth and may provide an alternative clinical strategy for remineralizing early enamel lesions.

Keywords: Bioactive glass; chlorhexidine; demineralization; dental caries; remineralization; white spot lesion

INTRODUCTION

Dental caries is a bacterial disease occurring as a result of continuous demineralization and remineralization in dental hard tissues. Poor oral hygiene and an increased

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acidic diet provide oral bacteria favorable environment to dissolute tooth constituents resulting in demineralization. Enamel demineralization results in prompt cavitation if not arrested or reversed at the precavitated stage.[1] The remineralization of enamel white spot lesion (WSL), the earliest clinical sign of caries manifesting as an intact surface zone and a porous subsurface, is a complex physiochemical process because the mineral crystals remaining in the lesion are less reactive.[1,2]

To avoid further demineralization, various methods have been implemented to promote the uptake of various ions such as calcium, phosphorus, and fluoride, into the tooth structure, thereby enhancing the remineralization of teeth.[3] In this study, newer strategies and materials have been compared to evaluate their remineralizing efficacy on artificial WSL.

Fluoride was the most commonly used remineralizing agent as it could enhance the formation of fluorapatite crystals, thereby preventing further demineralization. Furthermore, a synergistic effect was seen when fluoride was used in combination with other remineralizing materials.[2-4] Due to certain limitations such as occult caries and dental and skeletal fluorosis, newer remineralizing agents were introduced.[3] In this study, bioactive materials such as NovaMin, BioMin, and strontium-doped bioactive glass are used along with chlorhexidine (CHX) pretreatment on artificial WSLs.

Bioactive glass is a novel material that was introduced by Dr. Larry Hench in the 1960s. It acts as a biomimetic mineralizer matching the body's mineralizing traits and helps restoration of tissue structure and function.[5,6] Novamine is a bioactive glass which when exposed to an aqueous media provides calcium and phosphate ions forming a hydroxycarbonate apatite-like layer that is chemically similar to enamel and dentin. Biomine is a new bioactive glass that acts as a biomimetic mineralizer containing fluoride with high phosphate content and small particle size. It is found to diffuse into early enamel lesions providing a hydroxyapatite-like nucleation site for the formation of fluorohydroxyapatite.[7,8] Strontium has recently attracted some interest in caries management due to its similarity with calcium and cariostatic effect on dental caries. A new bioactive glass, strontium-doped bioactive glass (HX-BGC, SiO_2-P_2O_5-Na_2O-SrO), is introduced. In the presence of HX-BGC, the pH of the fluid was elevated, and the growth of cariogenic bacteria was inhibited. Furthermore, strontium acts synergistically with fluoride.[9-11] CHX is antiplaque, antigingivitic agent, antiseptic root canal irrigant, and also a remineralizing agent. It has been used for pretreatment to add upon synergistic effect in the remineralizing efficacy.[12-14]

The remineralizing efficacy of various agents used in the study was analyzed by assessing the mineral content on the tooth surface using energy-dispersive X-ray analysis (EDAX) analysis. The present study aimed to investigate the remineralizing effects of a strontium-doped bioactive glass, BioMin, and NovaMin on demineralized enamel and dentin.

MATERIALS AND METHODOLOGY

Preparation of samples

Twenty-four human premolars extracted for orthodontic purposes were selected for the study. Any caries, hypoplasia, developmental defects, restored, fractured, or periodontally diseased teeth are excluded. All the teeth were disinfected and preserved in a 10% formalin solution. Samples were decoronated at the cementoenamel junction using a diamond disc and stored in 0.1% thymol solution until the study initiated. Cylindrical molds were prepared and filled with chemical-cured resin. All the samples were embedded on the resin mold with the buccal surface facing upward, parallel to the horizontal plane.

A 4 mm × 4 mm enamel window was created in the center of all samples using adhesive tape and made acid-resistant by applying nail varnish around it. The adhesive tape was removed using an explorer after the samples were dried, exhibiting a rectangular area on the enamel surface where all the study samples were applied using a cotton applicator tip. This study involves treatment of Group A with strontium-doped bioactive glass (White lab, Saveetha Dental College and Hospitals, Chennai), Group B with biomine (Hydent Pro, Abbott India Ltd, Mumbai), Group C with novamine (SHY-NM, Group Pharma, India), and Group D with artificial saliva (Wet Mouth, ICPA Health Products Ltd, India) as control.

Synthesis of strontium-doped bioglass

Tetraethyl orthosilicate (TEOS, Sigma–Aldrich, 0.45 M) was mixed with ethanol and water in a ratio of 1:1. Concentrated nitric acid (45%) was added dropwise to the above mixture to initiate the gelation process, and the reaction was allowed to proceed for 12 h. Further, 0.06 M of orthophosphoric acid, 0.24 M of calcium nitrate, strontium chloride (0.05 M), and sodium hydroxide (0.19 M) were added individually at an interval of 1 h. Then, the reaction is allowed to proceed for overnight at 70°C. Further dried at 100°C for 24 h and sintered at 700°C for 6 h.

Synthesis of demineralizing and remineralizing solutions

The demineralizing solution was prepared at biochemistry laboratory, Saveetha Dental College and Hospitals, Chennai, using 2.2 mM potassium phosphate, 2.2 mM calcium chloride, and 0.05 M acetic acid, and pH was maintained at 4.8. Similarly, remineralizing solution was prepared using 0.9 mM sodium phosphate, 1.5 mM calcium chloride, and 0.15 M potassium chloride, and the pH was maintained at 7.0.

pH-cycling regime

A pH-cycling regime was conducted for 14 days to simulate the oral environment. This procedure was implemented twice daily (2 min per application) at 08:00 am and 06:00 pm. Each sample was first immersed in the demineralizing solution (5 mL per sample) for 10 min, followed by thorough rinsing with distilled water. Later, the samples were treated with respective remineralization agents, by continuously brushing onto the enamel window using a microbrush applicator tip, for 2 min. After that, they were rinsed under distilled water to remove the residual solution and incubated in remineralizing solution, which was refreshed every day at 37°C. After the pH-cycling regime, samples were thoroughly rinsed under running distilled water, followed by ultrasonically cleaning in distilled water for 5 min to remove any residual agent.

Procedure

After drying, baseline values were measured using EDAX. Samples were immersed in a demineralizing solution for 72 h to produce artificial WSLs. Demineralizing values were noted using EDAX. All demineralized specimens were pretreated with 0.2% CHX for 1 min. 0.0145 g sodium fluoride powder and 0.263 g HX-BGC powder were added into 5 mL deionized water to form a suspension of HX-BGC with fluoride which was applied in Group A. Groups B and C were treated with biomine and novamine dentifrice. Application of respective remineralizing agents was done in each group for 2 min. Artificial pH cycling is done to simulate the oral cavity. This procedure was done for 14 days [Figure I], twice a day, and EDAX analysis was done to record remineralized values

Statistical analysis

The data were analyzed using SPSS version 20.0. Continuous data were compared between the groups using a one-way ANOVA, followed by a *post hoc* test. The level of significance was set at $P < 0.05$.

RESULTS

Baseline, demineralized, and remineralized values obtained show that HX-BGC and CHX combination show maximum mineral gain compared to other groups [Table 1]. Furthermore, the values obtained were statistically significant suggesting all the materials used in the study have remineralization potential. Scanning electron microscopy and EDAX analysis of samples treated with HX-BGC and CHX combination showed the highest spike in calcium and phosphate ion regain compared to other ions [Figure II, Table 2].

DISCUSSION

Organic acids, by-products of carbohydrate fermentation, produced by cariogenic bacteria diffuse into the tooth subsurface causing demineralization.[1] Dissolution of minerals lowers the pH of the environment activating matrix metalloproteinases (MMPs), thereby causing collagen degradation and further tooth structure loss.[3] Remineralization is a natural repair process which mainly relies on the deposition of calcium phosphate ions from saliva to form a new layer on the surface. However, this natural phenomenon will be halted when there is a shift in demineralization–remineralization dynamics resulting in a lack of the integrity of hydroxyapatite (HA) latticework.[2]

Figure I: Study Setting I (a) Sample preparation, I (b) Artificial white spot lesion creation, I (c) Chlorhexidine pretreatment, I (d) Application of remineralizing agents, I (e) pH cycling, I (f) Energy-dispersive X-ray analysis

Fluoride has been the mainstay of enamel remineralization for decades. It controls caries through its topical effect inhibiting demineralization by forming fluorapatite on the enamel surface.[2,4] Fluorapatite is less soluble, therefore, increasing the enamel resistance to HA dissolution during acid attack. Formulations such as fluoride-containing varnishes, toothpaste, mouth rinses, solutions, gels, and orthodontic adhesives were used. This approach slowly declined due to occult caries, and dental and skeletal fluorosis.[4,5] Use of agents such as casein polyphosphate-amorphous calcium phosphate, novamine, nanohydroxyapatite, and newer bioactive glass materials was introduced. Furthermore, a synergistic effect was observed when fluoride was used with other remineralizing agents.[5] Hence, in this study, fluoride was chosen to be adjunct to the HX-BGC solution, and also, it is also present in biomine dentifrice.[6]

Recently, a bioactive glass (45S5) has been developed and is found to remineralize WSLs. It aids in HA formation when brought into contact with saliva. Fluoride-containing glasses have "smart" properties, with increased remineralization activity in low-pH environments.[7,8] In this study, newer bioactive glass materials such as novamine, biomine, and strontium-doped bioactive glass were used. Bioactive glass aids in remineralization by releasing calcium and phosphate ions into the oral cavity, and these supersaturated ions precipitate on the dental hard tissues.[8,9] The presence of mineral residues on the demineralized tooth surface serves as nuclei for apatite formation and also an abundant amount of calcium and phosphate ions in the surrounding environment can promote this remineralization activity. In addition, intact dentin collagen acts as a scaffold for minerals to grow on.[12] Hence, in this study, a favorable environment was created by adopting pH-cycling regime that could continuously favor the precipitation of calcium phosphate ions, thereby enhancing apatite formation on the demineralized tooth surface.

Table 1: Mean calcium and phosphorus ratio values obtained at baseline, demineralization, and remineralization

	Baseline, mean±SD	Demineralization, mean±SD	Remineralization, mean±SD
Group A	2.42±0.14	2.19±0.34	2.32±0.14
Group B	2.37±0.20	2.12±0.17	2.23±0.24
Group C	2.18±0.22	2.08±0.17	2.10±0.10
Group D	2.14±0.19	2.08±0.24	2.09±0.24

SD: Standard deviation

Table 2: Multiple comparisons of mean calcium and phosphate ratio after remineralization between the groups

Groups	Remineralization, mean±SD	Comparison	*P*
Group A	2.32±0.14	Group A with Group B, Group C, Group D	0.03*
Group B	2.23±0.24	Group B with Group A, Group C, Group D	0.03*
Group C	2.10±0.10	Group C with Group A, Group B, Group D	0.04*
Group D	2.09±0.24	Group D with Group A, Group B, Group C	0.04*

* $p<0.05$. SD: Standard deviation

CHX is biguanide with enhanced antibacterial properties and also remineralizing effect recently.[12,13] It acts by electrostatic interaction, i. e., being positively charged, and it binds to negatively charged pellicle and biofilm layer and exhibits an antibacterial effect for a long time. This substantivity nature of CHX maintains sound collagen cross-linkage by inhibiting MMP activity. Furthermore, it forms a stable calcium-CHX complex which stimulates new HA-forming crystals from other remineralizing agents.[12] It is available in various concentrations such as 0.2%, 2%, and 20%.[13] Hence, in this study, 0.2% CHX solution was used

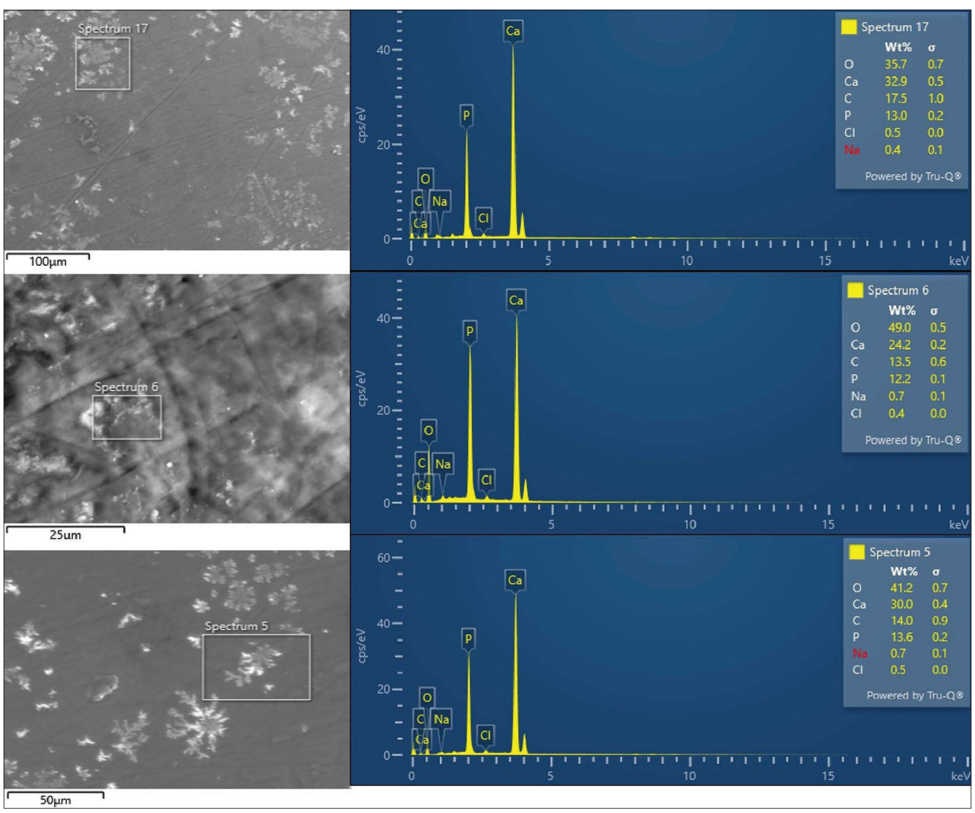

Figure II: SEM images and energy-dispersive X-ray analysis elemental analysis of a sample from Group A – strontium-doped bioactive glass as pretreatment agent for 2 min in each sample according to the American Dental Association guidelines for brushing time.

HX-BGC and fluoride combination has proven effects on apatite crystallization.[14,15] Strontium has a chemical similarity to calcium; hence, it can be incorporated into the crystal lattice of HA. Furthermore, it has reduced particle size than hydroxyl ion which aids in easy diffusion and penetration into crystal lattice. Moreover, the release of fluoride from bioactive glass is increased in the presence of strontium, thereby enhancing the bioactivity and chemical stability of the newly formed apatite.[16] In this study, HX-BGC when used in combination with fluoride showed superior mineral regain than other groups.

Biomine is a fluoride-containing bioglass that contains calcium fluorophosphosilicate. It showed superior remineralization values than novamine when used in combination with CHX in this study which was in line with Shailk *et al*.[10] This would be because biomine has got smaller particle size which can easily diffuse into the crystal lattice, and it has three times higher phosphate content than NovaMin which can dissolve and stay up to 12 h. NovaMin is calcium sodium phosphosilicate glass that releases sodium, calcium, and phosphate ions, thereby increasing the pH of the surrounding environment. It aids in calcium phosphate precipitation which mineralizes to form mature carbonate-enriched hydroxycarbonate apatite layer.[17] In this study, statistically significant EDAX values were obtained for all the experimental groups suggesting that all these materials have remineralization potential. CHX with novamine combination showed values lesser than HX-BGC, and biomine combination suggesting fluoride gives synergistic effect when used along with bioglass-based technology. Under certain limits of the study, further research should be done to evaluate the remineraliszation potential *in vivo* conditions.

CONCLUSION

Within the conditions of the study,

- Bioglass-based dentifrices have a synergistic effect with CHX pretreatment on demineralized dental hard tissues
- Fluoride has a synergistic effect when used along with other remineralizing agents
- The new strontium-doped bioactive glass (HX-BGC) can reduce mineral loss and promote remineralization through precipitation of newly formed apatite
- HX-BGC with CHX has higher remineralized values when compared with BioMin and NovaMin.

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Conflicts of interest

There are no conflicts of interest.

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