Comparative evaluation of qualitative and quantitative remineralization potential of four different remineralizing agents in enamel using energy-dispersive X-ray: An in vitro study

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Abstract

Background: The principles of minimally invasive dentistry clearly tell us the need for clinically effective measures to remineralize the early enamel caries lesions.

Aims and Objectives: The aim of this study was to evaluate the remineralisation potential of four different remineralisation agents used quantitatively by surface microhardness and qualitatively by energy dispersive X-ray analysis.

Materials and Methods: Artificial enamel lesions were created on the buccal surfaces of 60 extracted mandibular second premolar. Specimens were randomly assigned to four groups (n=15) according to the remineralisation agents used: Group 1-Nano Hydroxyapatite, Group 2-Fluoride, Group 3-CPP ACP, Group 4-Chitosan 5mg. All products were applied according to the manufacturer’s instructions and specimens were stored in daily renewed artificial saliva. Surface microhardness was assessed using Rockwell hardness test and change in mineral content was evaluated using Energy Dispersive X-ray analysis.

Statistical Analysis: One way analysis of Variance test and post-hoc Tukey test were conducted for multiple group comparison.

Results: There was remarkable remineralisation in Hydroxyapatite treated comparatively to the other three groups.

Conclusion: All remineralising agents showed improved surface remineralisation. However complete remineralisation did not occur within 7 days. Nanohydroxyapatite showed the highest potential for remineralisation followed by CPP-ACP, Chitosan and Fluoride.

Keywords: Enamel remineralisation, Caries prevention, Critical pH, Artificial saliva

INTRODUCTION

Demineralization is the process of removing mineral ions from the hydroxyapatite crystals of hard tissue by a regular exposure of tooth enamel to acids produced by bacterial plaque. Demineralization occurs from a complex chemistry between bacteria, diet, and salivary components. A drop in the pH of oral cavity results in demineralization. When the bacterial colony in the biofilm continues to produce acids with sugar consumption, pH falls to 4.5–5 from the normal pH that is 7 and the minerals are dissolved. This results in formation of pores between the mineral structures Critical
pH is the highest pH at which there is a net loss of minerals from tooth enamel. At this pH, saliva and plaque fluid cease to be saturated with calcium and phosphate, thereby permitting hydroxyapatite to dissolve. The hydroxyapatite has critical pH around 5.5, below which demineralization occurs while remineralization occurs above this critical pH. During the demineralization process, acid diffuses between the rods and reaches deeper areas of the enamel and into dentin. The carbonated hydroxyapatite crystals are more susceptible to dissolution. The calcium and phosphate ions that are lost from the tooth diffuse out into dental plaque fluid and saliva. If the acid attack is chronic and prolonged, progressively greater amounts of calcium and phosphate minerals diffuse out of the tooth, causing the crystalline structure of the tooth to shrink in size, while pores enlarge. Eventually, a carious lesion develops.[2]

Remineralization is the process of bringing minerals from the surrounding environment into partially demineralized tooth structure. Remineralization of carious lesion is naturally achieved by salivary ions and it can be enhanced by external factors or elements such as fluoride and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP).[3] During remineralization, the growth of newly formed crystal fluor-hydroxyapatite takes place, and with advancing growth, crystals fuse with each other to form large crystals with hexagonal outline. Therefore, the best strategy for caries management is to focus on methods of improving remineralization process with the aid of remineralizing agents. Commercially, a variety of remineralizing agents are available. Some of them are nano-hydroxyapatite, chitosan, CPP-ACP, CPP-ACP fluoride, functionalized beta-tricalcium phosphate, bioactive glass-containing sodium phosphosilicate, sodium trimetaphosphate, biofilm modifiers (arginine, triclosan, and xylitol), and self-assembling peptides.[4] Hence, the aim of this study was to evaluate the remineralizing efficacy of four different agents using surface microhardness analysis and energy-dispersive X-ray (EDAX) analysis.

**MATERIALS AND METHODS**

**Specimen preparation**
A total of sixty sound freshly extracted lower second premolars for orthodontic purpose were taken up for the study. Teeth with any visible caries or white spot lesions were excluded from the study.

The teeth were decoronated using a double-faced diamond disc, and the crown was embedded in self-cured acrylic resin mold with buccal surface facing upward. The buccal surfaces were polished.

**Demineralization**
The specimens were subjected to demineralization process artificially by immersing the specimens in a custom-made demineralizing solution. The solution contained 2.2 mM calcium chloride, 2.2 mM sodium dihydrogen orthophosphate dehydrate, and 0.05M acetic acid. The pH was adjusted to 4.4 with 1M potassium hydroxide. Each group was immersed separately in a daily renewed demineralizing solution for 7 consecutive days at 37°C in incubator until artificial demineralized lesion was created. Specimens were then stored in distilled deionized water.

**Storage of specimens**
Artificial saliva was prepared according to the formulation of Ten Cate et al. and Duijsters et al., which contained 1.5 mM calcium chloride, 0.9 mM sodium dihydrogen orthophosphate dehydrate, and 0.15M potassium chloride which had a pH of 7.

**Materials**
Four remineralizing agents were tested in this study: nano-hydroxyapatite (Remin Pro, Voco GmbH, Germany), fluoride (Fluor Protector Gel, Ivoclar Vivadent, Liechtenstein, Germany), CPP-ACP (GC Tooth Mousse, GC America Inc., USA), and chitosan 5 mg (Bangalore Fine Chemicals, India) [Figure 1].

**Remineralization**
Specimens were randomly divided into four groups of n = 60:
- Group 1: Nano-hydroxyapatite (n = 15)

![Figure 1: Armamentarium of material used in this study](image-url)
Hemalatha, et al.: Qualitative and quantitative remineralization potential of four different agents in enamel

- Group 2: Fluoride \( (n = 15) \)
- Group 3: CPP-ACP \( (n = 15) \)
- Group 4: Chitosan 5 mg \( (n = 15) \).

Samples were dried and agents were applied according to manufacturer’s instructions and kept undisturbed for 5 min at every 24 h for 7 days. The specimens were then stored in artificial saliva. Artificial saliva was renewed every 24 h just before immersion of freshly treated samples.

The remineralization capability of the four materials tested was evaluated based on:

a. Surface microhardness (SMH) which determines the ability of the materials to withstand the force without distortion

b. Scanning electron microscopy (SEM)-EDAX which determines the changes in mineral content in demineralized and remineralized area comparatively.

**Assessment of surface microhardness**

SMH was measured at baseline of sound enamel, after demineralization, after 1 week of remineralization. SMH was performed using a Rockwell Hardness Tester. Each measurement was carried out by applying 60 Kgf load for 20 s oriented perpendicularly to the enamel surface to measure the depth of penetration. All readings were performed by the same examiner using the same calibrated machine. In each reading, three indentations were made and their average was taken to represent the specimen’s hardness value.

**Analysis of mineral content**

Samples were subjected to SEM-EDAX analysis and the values of mineral content (wt %) were recorded. SEM-EDAX analysis of the samples before demineralization and after remineralization was evaluated which showed deposition of minerals and increase in the mineral content after application of the remineralizing agent. However, the deposited mineral content varied among groups and hence the remineralization potential.

**Statistical analysis**

The SMH of samples was compared across study groups. The mean and standard deviation of microhardness of samples were obtained for each group, and comparison was performed using one-way ANOVA. The pair-wise comparison of mean microhardness between groups was carried out using Tukey’s post hoc test. The statistical significance was performed using IBM® SPSS® (version 20) software (SPSS Inc., IBM Corporation, NY, USA).

Differences in mean Ca:P between the groups for sound enamel and remineralized enamel were analyzed by ANOVA followed by post hoc Tukey’s pair-wise comparison test.

**RESULTS**

In the present study, evaluation of remineralizing effect of fluoride, CPP-ACP, chitosan 5 mg, and nano-hydroxyapatite was done using EDAX. EDAX is considered the gold standard for the evaluation of mineral loss or gain in experimentally induced initial caries lesions. It is a microanalytical technique used in conjunction with SEM, which does the structural analysis.

When deposition of minerals was compared between four groups, it was found that nano-hydroxyapatite has achieved the highest rate of deposition [Table 1]. The deposition of calcium and phosphate is more in the nano-hydroxyapatite group as its composition mainly has calcium and phosphate in it followed by CPP-ACP which has ACP followed by chitosan, a deacetylated polymer of chitin which possesses highly reactive amino (-NH2) and hydroxyl groups (OH-) and fluoride which forms fluorohydroxyapatite. The comparison of four these remineralising agents in Energy-dispersive X-ray spectroscopy is illustrated in [Figure 2]. Hardness is directly proportional to deposition of calcium and phosphate. In this study surface, microhardness was assessed using Rockwell hardness test by analyzing the depth of penetration. In this study, nano-hydroxyapatite was found to be superior among other materials.

**DISCUSSION**

An important goal of modern dentistry is to manage noncavitated carious lesions noninvasively through remineralization, thereby preventing disease progression and improving strength, esthetics, and function of teeth. A fundamental to this therapeutic philosophy is the need for new and highly efficacious technologies for enamel remineralization, which resulted in the evolution of various contemporary remineralizing agents.[4,5]

Many methods are used to provide evidence of mineral loss or gain. SMH analyses have been broadly used to assess the demineralization and remineralization changes that occur in the enamel. SMH evaluations are quite simple, fast, and easy to measure nondestructively. SMH is used Energydispersive Xray spectroscopy of four different remineralizing agents is shown in Figure 2. for measuring the resistance of materials against plastic deformation from a standard source allowing repeated measurements of the same specimen over a period of time. Thus SMH evaluations are considered a suitable choice to estimate mineral changes. [5,10]

The commonly used microhardness tests for evaluating enamel remineralization are Vickers and Knoop microhardness tests. In this study, Rockwell hardness test was used to analyze the depth of penetration. When the depth of penetration is less, it is well understood
Nanoparticulate hydroxyapatite has the ability to enhance the penetration of its crystals through interprismatic spaces of the enamel, thus resulting in the formation of hydroxyapatite crystals. The demineralized enamel surface was considerably rough and slightly porous. This facilitates the nano-hydroxyapatite to penetrate into the interprismatic spaces due to precipitation process. In addition, it also attracts a large amount of Ca²⁺ and (PO₄)³⁻ from the saturated solution at the outer layer of enamel surface to refill the vacant positions of the crystals. These were the suggested mechanism by which nano-hydroxyapatite was found to be more effective to the highest deposition of calcium and phosphate crystals comparatively, thus enhancing the remineralization potential in relation to all the other materials.

Tschooppe et al. evaluated the effects of a toothpaste-containing nano-hydroxyapatite on the enamel and dentin remineralization and showed that NHA toothpaste had greater efficacy for enamel and dentin remineralization than amine fluoride toothpaste. Bajaj et al. compared CPP-ACP, tricalcium phosphate, and hydroxyapatite on remineralization of artificial caries-like lesions on primary enamel, which showed that hydroxyapatite had better remineralization efficacy compared to CPP-ACP and tricalcium phosphate. In an in vitro study, nano-hydroxyapatite caused remineralization of the early caries lesions. Nano-hydroxyapatite-containing paste has been shown clinically to decrease the duration of postbleaching sensitivity which is the most common side effect of the bleaching process.

Seven-day remineralization failed to remineralize artificial enamel caries completely. It is one of the drawbacks observed in the study, and hence, the period of application for complete remineralization cannot be determined for all the remineralizing agents used. Complete remineralization did not occur within the time span of 7 days.

Till date, it was difficult to deal with the white spot lesions specifically in the esthetic zones. It either included noninvasive treatment with fluorides or invasive approach with tooth-colored restorative materials. However, considering the disadvantages with both the techniques, the use of micro-invasive approach has shown promising results in immediate masking of the demineralized areas, thus improving the esthetics.

**Limitation**

As the study is limited to in vitro conditions, further clinical trials are necessary to assess the remineralization potential and longevity of nano-hydroxyapatite. As in vitro conditions may be different when compared to the in vivo with dynamic complex biological system, after further research and clinical trials, with proper case selection, nano-hydroxyapatite may be used as one of the methods to enhance the remineralization. However, in the present clinical scenario, nano-hydroxyapatite can be used as material to limit the demineralization, as it has the ability to revert back the demineralized enamel to sound enamel.

**CONCLUSION**

Within the limitations of the study, it can be concluded that:

1. All remineralizing agents showed improved surface remineralization
2. Nano-hydroxyapatite effectively remineralizes initial enamel caries
3. Nano-hydroxyapatite can be used as an effective tool for remineralization of early enamel caries.

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**Conflicts of interest**
There are no conflicts of interest.

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