

Comparative Evaluation of Remineralizing Potential of a Paste Containing Bioactive Glass and a Topical Cream Containing Casein Phosphopeptide-Amorphous Calcium Phosphate: An in Vitro Study

Ratheesh Rajendran¹, Radhakrishnan Nair Kunjusankaran², Raghu Sandhya³, Aadit Anilkumar⁴, Rakhi Santhosh⁵, Santosh Rayagouda Patil⁶

¹Department of Conservative Dentistry and Endodontics, Saveetha Dental College, Saveetha Institute of Medical and Technical Science, Saveetha University, Chennai, India.  0000-0001-7151-3573

²Department of Conservative Dentistry and Endodontics, Azeezia College of Dental Sciences and Research, Kerala, India.

 0000-0002-7658-3404

³Department of Conservative Dentistry and Endodontics, Saveetha Dental College, Saveetha Institute of Medical and Technical Science, Saveetha University, Chennai, India.  0000-0002-8089-2226

⁴Department of Conservative Dentistry and Endodontics, Azeezia College of Dental Sciences and Research, Kerala, India.

 0000-0002-9440-8714

⁵Department of Conservative Dentistry and Endodontics, Azeezia College of Dental Sciences and Research, Kerala, India.

 0000-0001-9880-2711

⁶Department of Oral Medicine and Radiology, Saveetha Dental College, Saveetha Institute of Medical and Technical Science, Saveetha University, Chennai, India.  0000-0003-0715-497X

Author to whom correspondence should be addressed: Dr. Ratheesh R., Department of Conservative Dentistry and Endodontics, Saveetha Dental College, Saveetha Institute of Medical and Technical Science, Saveetha University, Chennai, India. Phone: +91 8281318181. E-mail: dr.ratheesh@hotmail.com.

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Abstract

Objective: To evaluate and compare the remineralization potential of a dentifrice containing bioactive glass and a topical cream containing casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) in remineralizing artificial carious lesion on enamel. **Material and Methods:** Forty-five freshly extracted human permanent premolar teeth were selected. Samples were divided into three groups: GI - regular tooth paste without specific remineralizing agent; GII - tooth paste containing calcium sodium-phosphosilicate (novamin) and GIII - topical cream containing casein phosphopeptide-amorphous calcium phosphate. All the sound enamel samples were viewed under scanning electron microscope (SEM) to assess the topographical pictures of enamel surface and energy dispersing x-ray analysis (EDAX) was done to estimate quantitatively the amounts of mineral (calcium and phosphorous). The mineral content of calcium and phosphorus after demineralization in each group was noted. The samples were then subjected to SEM and EDAX. **Results:** GI does not show any increase in the calcium and phosphorus after applying toothpaste without any remineralizing agent but GII and GIII showed a net increase in calcium and phosphorous values after applying concern-remineralizing agents. Inter group comparison showed GIII yield higher net calcium and phosphorous values than GII. **Conclusion:** Two remineralizing agents showed remineralization potential on enamel surfaces. Casein phosphopeptide-amorphous calcium phosphate showed better remineralizing potential than calcium sodium phosphosilicate. Hence CPP-ACP can be considered as the material of choice in remineralizing early enamel carious lesions.

Keywords: Phosphopeptides; Calcium Phosphates; Caseins; Tooth Remineralization.

Introduction

Dental caries is the most common cause for the loss of enamel in a clinical situation. Dental caries is easily detectable and reversible at an early stage. Once the incipient lesion proceeds to cavitation, the condition becomes irreversible. Hence it is necessary to prevent the progression of dental caries at an early stage, rather than to develop treatment strategies for progressive dental caries. Enamel is subjected to innumerable cycles of demineralization and remineralization throughout its lifetime, which controls progression or reversal of caries.

Recently, various remineralizing agents have been introduced, most of which contain fluoride, calcium, phosphate ions in varied forms and concentrations. Application of remineralizing agents to the tooth structure aim at controlling the demineralization /remineralization cycle, depending upon the microenvironment in around the tooth [1].

There are different remineralizing agents incorporating casein phosphopeptide-amorphous calcium phosphate and bioactive glass (novamin), both of which are widely used for remineralization. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) contains nanocomplexes of milkprotein casein with ACP. It promotes remineralization of the carious lesions by maintaining a supersaturated state of essential minerals, at the same time it also hinders colonization of dental surfaces by cariogenic bacteria. Anticariogenic mechanism of CPP-ACP is the incorporation of nanocomplexes into plaque and on to tooth surfaces [2].

Novamin (calcium sodium phosphosilicate) is a bioactive glass and were originally developed as bone regenerative materials [3]. These materials are reactive when exposed to body fluids and deposit hydroxycarbonate apatite [4], a mineral that is chemically similar to natural tooth minerals. Novamin adheres to an exposed dentin surface and reacts with it to form a mineralized layer. The layer formed is resistant to acid challenges and is mechanically strong.

This study was done to evaluate and quantitatively compare the remineralizing potential of a commercially available dentifrice containing novamin and a topical cream containing casein phosphopeptide-amorphous calcium phosphate against a regular tooth paste without any specific remineralizing agents using scanning electron microscope (SEM) equipped with Energy dispersing X-ray analysis (EDX).

Material and Methods

Forty-five freshly extracted human permanent premolar teeth (extracted for orthodontic reasons) were collected for the study after obtaining written informed consent from the patients. The teeth selected for this study were free from dental caries, restorations or developmental defects.

The coronal portion of the teeth were sectioned horizontally using a diamond disc, with a slow speed straight hand piece at the level of CEJ and forty-five enamel specimen of 4 x 4 x 1 mm in size, were prepared from the buccal surfaces of the teeth. Forty-five enamel samples were divided into three groups of 15 each based on the toothpaste applied on the tooth surface (Table1).

Table 1. Distribution of the groups according to the remineralizing agent.

Groups	Remineralizing Agent
Group I (n=15)	Regular tooth paste without specific remineralizing agent
Group II (n=15)	Tooth paste containing calcium sodium-phosphosilicate (Novamin)
Group III (n=15)	Topical cream containing casein phosphopeptide-amorphous calcium phosphate

All the sound enamel samples were viewed under scanning electron microscope to assess the topographical pictures of enamel surface and energy dispersing X-ray analysis was done to estimate quantitatively the amounts of mineral (calcium and phosphorous) in all the three groups.

Demineralization of Samples

All specimens from group I, II and III were subjected to demineralization by placing in McInnes demineralizing solution. The solution was freshly prepared before use [5]. Each sample was subject to two cycles of demineralization. The demineralizing agent prepared was applied to the surface of enamel samples using a cotton applicator for five minutes. It was then washed under running tap water, damped dry with absorbent paper and then stored in artificial saliva for 24 hours to prevent dehydration. The second cycle of demineralization was done in a similar manner after 24 hours.

All these specimens were subjected to scanning electron microscopy and energy dispersing X-ray analysis. The mineral content of calcium and phosphorus after demineralization in each group was noted.

Remineralization of Samples

In Group I, enamel samples were treated with regular tooth paste without specific remineralizing agent and acted as control group. Group II was treated with tooth paste containing calcium sodium phosphosilicate (novamin) and Group III treated with topical cream containing casein phosphopeptide-amorphous calcium phosphate

In each group the corresponding material was applied on the demineralized samples with an electronic toothbrush for three minutes twice daily (12 hour interval). The samples were then gently rinsed with water, stored in artificial saliva. This procedure was repeated for 28 days. The samples were then subjected to scanning electron microscope to assess the topographical pictures and the surface changes seen on enamel and energy dispersing X-ray analysis to estimate quantitatively the amounts of mineral in a given tooth sample after the remineralizing cycle.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics for Windows Software, version 22 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to calculate the mean and standard deviation. Kruskal-Wallis, ANOVA and Mann-Whitney tests were used. The level of significance was set at 5%.

Ethical Aspects

This research was approved by the Ethics Research Committee of the Saveetha Dental College, Saveetha Institute of Medical and Technical Science, Saveetha University.

Results

Group I showed a mean calcium and phosphorus value of 65.32 ± 0.51 and 21.13 ± 0.75 , respectively for sound enamel. After remineralization with regular toothpaste the mean calcium and phosphorus values obtained were 55.24 ± 0.49 and 15.76 ± 0.54 respectively which is lesser than that of sound enamel and was statistically significant ($p < 0.001$). The values obtained after remineralization were similar to the mean calcium and phosphorus content of demineralised samples (Table 2).

Table 2. Comparison of mean calcium and phosphorus of normal toothpaste.

Condition	Calcium	Phosphorus
	Mean (SD)	Mean (SD)
Baseline (B)	65.32 ± 0.51	21.13 ± 0.75
Demineralized (D)	55.15 ± 0.51	15.75 ± 0.59
Remineralized (R)	55.24 ± 0.49	15.76 ± 0.54
p-value	0.000	0.000
Mann-Whitney test	B>D=R	B>D=R

Group II showed a mean calcium and phosphorus value of 65.27 ± 0.58 and 20.79 ± 0.78 , respectively for sound enamel. Demineralized samples showed a mean calcium and phosphorus value of 55.09 ± 0.54 and 15.71 ± 0.57 , respectively. After remineralization of the demineralized specimen with novamin containing tooth paste the mean calcium and phosphorus values obtained were 61.29 ± 0.62 and 17.56 ± 0.43 respectively, which is higher than the demineralized samples and was statistically significant ($p < 0.001$) (Table 3).

Table 3. Comparison of mean calcium and phosphorus of novamin.

Condition	Calcium	Phosphorus
	Mean (SD)	Mean (SD)
Baseline (B)	65.27 ± 0.58	20.79 ± 0.78
Demineralized (D)	55.09 ± 0.54	15.71 ± 0.57
Remineralized (R)	61.29 ± 0.62	17.56 ± 0.43
p-value	0.000	0.000
Mann-Whitney test	B>R>D	B>R>D

Group III showed a mean calcium and phosphorus value of 65.32 ± 0.66 and 20.89 ± 0.76 respectively for sound enamel. Demineralized specimen showed a mean calcium and phosphorus content of 55.22 ± 0.47 and 15.69 ± 0.53 , respectively. After remineralization with CPP-ACP containing toothpaste the values obtained were 63.05 ± 0.37 and 18.54 ± 0.39 respectively, which is higher than the values obtained for demineralized samples and was statistically significant ($p < 0.001$). (Table 4).

Table 4. Comparison of mean calcium and phosphorous of GC Tooth Mousse.

Condition	Calcium Mean (SD)	Phosphorous Mean (SD)
Baseline (B)	65.32 ± 0.66	20.89 ± 0.76
Demineralized (D)	55.22 ± 0.47	15.69 ± 0.53
Remineralized (R)	63.05 ± 0.37	18.54 ± 0.39
p-value	0.000	0.000
Mann-Whitney test	B>R>D	B>R>D

Intergroup comparison showed statistically significant differences in the mean calcium and phosphorus levels, with group III yielding higher mean calcium and phosphorus levels and group I showing least value (Table 5).

Table 5. Comparison of mean calcium and phosphorous of three pastes (remineralized).

Groups	Calcium Mean (SD)	Phosphorous Mean (SD)
Normal Toothpaste	55.24 ± 0.49	15.76 ± 0.54
Novamin	61.29 ± 0.62	17.56 ± 0.43
GC Tooth Mousse	63.05 ± 0.37	18.54 ± 0.39
p-value	0.000	0.000
Mann-Whitney test	3>2>1	3>2>1

Discussion

Clinically the earliest detectable sign of dental caries is the incipient enamel lesion or white spot, which becomes detectable radiographically only as the lesion progresses into the enamel and dentin [6]. The lesion appears opaque as a result of loss of subsurface enamel, resulting in loss of enamel translucency [7]. At this stage, before cavitation, therapeutic measures can be applied to reverse or arrest the progression of the lesion [6]. If left untreated, a cavitated carious lesion can develop. Early diagnosis with newer caries detection devices enables small lesions to be identified so that remineralization of lesions by preventive measures can be attempted. Applying strategies to control, arrest, or reverse the disease process can reduce the economic burden, pain, and suffering of placing and replacing restorations.

Organic acids, produced by acidogenic bacteria, diffuse through the enamel and dentin organic matrix into underlying tissues [8,9]. When the acid reaches a susceptible site on the crystal surface, minerals dissolve into the surrounding aqueous phase. Demineralization of enamel occurs at a critical pH of about 5.5. If the calcium and phosphate supersaturation levels are restored, minerals will diffuse into the tooth and deposit a new, more acid-resistant layer on the crystal remnants in the non-cavitated lesion [10]. It has been noted that the outermost enamel layer is the most resistant to dissolution [11]. Two mechanisms have been proposed for the formation of this hypermineralized surface layer on incipient lesions. The first is the deposition of fluoride and other ions from saliva and the other is the outward diffusion of minerals and ions from the subsurface lesion that would be deposited in the surface layer [11,12].

In this study in order to simulate an incipient caries process demineralization of the surface enamel layer of the specimens in the experimental groups was done using the Mc Innes solution as it was an accepted protocol [5]. Demineralization with McInne's solution was done in two cycles as it has been proved in the previous study that only after two cycles of demineralization was there a significant reduction in microhardness of enamel [5]. Specimens were kept in artificial saliva to simulate oral environment.

Remineralization helps in regaining the lost calcium, phosphate, and fluoride ions of the tooth structure and is replaced in the form of fluorapatite crystals [13]. In the development of dental caries, the relationship between demineralization and remineralization is influenced by the presence of saliva, which facilitates the transportation of these ions to the exposed surfaces of teeth [13]. Saliva contains a supersaturated solution of calcium and phosphate, which neutralizes acids.

Fluoride which is considered as an age old anti carious agent, has an antimicrobial effect on the bacteria and plays a role in remineralization and creating acid-resistant tooth structure. Low-dose fluoride (1,000 to 11,100 ppm) in dentifrices, and fluoride mouth rinses has been reported to reduce caries by maintaining a low concentration of salivary fluoride available for remineralization with daily uses. Topical fluoride in the form of acidulated phosphate fluoride (1.23%) and varnishes have also been used. Though fluoride is considered to be the gold standard active agent in anti-caries toothpastes [14] as the severity of the plaque acid attack increases, the pH drops down to about 4.5 [15] and fluoride action becomes ineffective in controlling the lesion progression.

Newer technologies are now being used for remineralization through various means by providing calcium and phosphate into saliva, thereby changing the ratio towards remineralization. There are various remineralizing agents available such as tricalciumphosphate, calcium sodium-phosphosilicate (novamin), casein phosphopeptide-amorphous calcium phosphate that are incorporated either into a toothpaste or a topical cream. Tricalcium phosphate is a new hybrid material that fuses beta tricalcium phosphate (β -TCP) and sodium lauryl sulfate or fumaric acid. This blending results in "functionalized" calcium and a "free" phosphate, designed to increase the efficacy of fluoride remineralization. β -TCP is similar to apatite structure and possesses unique calcium environments capable of reacting with fluoride and enamel [16]. Disadvantage of this paste is the partial or complete disintegration or exhibition of inhomogeneous behaviour as soon as it comes into contact with body fluids. The paste readily separates during extrusion from syringes, the more liquid part being forced out of the syringe while the more solid part remains in the syringe.

Novamin which is a bioactive glass, contains calcium, sodium, phosphorous, and silica ions and when used in the form of a tooth paste, binds to the tooth surface and continuously deposits a natural crystalline hydroxyl carbonate [7]. It acts as a biomimetic mineralizer, matching the human body's own mineralizing traits [17]. In the aqueous environment around the tooth, i.e., saliva in the oral cavity, sodium ions from the Bioactive Glass particles rapidly exchange with hydrogen cat ions (in the form of H_3O^+) and this brings about the release of calcium and phosphate ions from the glass. A localized, transient increase in pH occurs during the initial exposure of the material to water

due to the release of sodium. This increase in pH helps to precipitate the extra calcium and phosphate ions provided by the Bioactive Glass to form a calcium phosphate layer. As these reactions continue, this layer crystallizes into hydroxycarbonate apatite (HCA) [18]. The combination of the residual Novamin particles and the newly formed HCA layer results in remineralization of the enamel surface and prevents further demineralization.

In the present study, a toothpaste containing bioactive glass (novamin) was used for the purpose of remineralization in Group II. Group III was treated with Topical cream containing casein phosphopeptide-amorphous calcium phosphate. CPP-ACP nanocomplexes have been proven to be efficacious in both the prevention and reversal of enamel lesions in caries models. It has been shown that CPP-ACP can be used to prevent demineralization and promote remineralization of early enamel lesions. Studies have shown a short term remineralizing effect in clinical in situ trials and long-term caries-preventing effect in the in vivo randomized control trial [19,20]. The CPP has been shown not only to stabilize ACP, but also to deliver and localize ACP at the tooth surface [21]. The proposed anticariogenic mechanism for CPP-ACP is by the localization of amorphous calcium phosphate on the tooth surface, which buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to the tooth enamel and thus preventing demineralization and enhancing remineralization.

Modern prospective caries studies require the measurement of even small changes in tooth mineral content. EDX is considered as the “gold standard” for the determination of mineral loss or gain in experimentally induced initial carious lesions. The EDX provides a very precise quantitative measurement of the mineral content by elemental analysis at the ultrastructural level [22,23]. It is a micro analytical technique used in conjunction with SEM wherein SEM does the structural analysis and the elemental analysis is done by EDX.

In this study, Group I (control group treated with regular tooth paste) showed mean calcium and phosphorous levels of 55.24 ± 0.49 and 15.76 ± 0.54 respectively after remineralization. Group II in which samples were treated with novamin containing paste showed mean calcium and phosphorous levels of 61.23 ± 0.65 and 17.52 ± 0.42 after remineralization. There was a net increase in calcium and phosphorous content after remineralization in Group II compared to same samples after demineralization. The net increase was found to be 6.05% for calcium and 1.75 % for phosphorous.

The result obtained in this research is in accordance with previous study [24], having been observed that bioactive glass containing remineralization tooth paste showed significant remineralizing potential in inhibition of artificial enamel sub-surface lesion after 10 days of remineralization phase. A similar study also concluded that bioactive glass had a significant remineralizing potential [25]. Another authors showed that novamin resulted in the formation of a protective layer on the enamel surface [26].

Group III treated with CPP-ACP had a mean calcium and phosphorous levels of 63.09 ± 0.38 and 18.52 ± 0.44 after remineralization. Group III also showed a net increase in calcium and

phosphorous content after remineralization. The net increase was found to be 7.91% for calcium and 2.75% for phosphorous.

CPP-ACP had remineralizing effect on artificial sub surface enamel lesions and the remineralizing effect increased with increase in the usage of paste on first, fifth and tenth day respectively [27]. There was no regression of artificial caries lesions on usage of CPP-ACP, while remineralization occurred using micro abrasion technique [28]. This may be due to the difference in estimating the mineral content in a different method. They used Quantitative Laser Fluorescence method, which was found to be less technique sensitive compared to SEM EDAX.

Results of this study showed that Novamin and CPP-ACP are capable of remineralizing the demineralized artificial caries lesion. These changes are found to be statistically significant. Intergroup comparison between groups II (novamin) and III (CPP-ACP) showed that group III had higher mean calcium and phosphorus levels than group II and was statistically significant. The results of the present study showed that CPP-ACP has the better remineralizing potential than calcium sodium phosphosilicate (Novamin). A similar study evaluated the remineralizing potential of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), 0.21% sodium fluoride - tricalcium phosphate (f-TCP) and calcium sodium phosphosilicate (CSP) containing tooth pastes on demineralized tooth surfaces using micro CT and microhardness test and concluded that CPP-ACP showed the better remineralizing potential than the other two agents [29].

Due to the peculiar nature of CPP, by stabilizing calcium phosphate in a metastable solution, it facilitates high concentrations of calcium and phosphate ions, including CaHPO_4 , which can diffuse into the enamel subsurface lesion. The CPP will also maintain the high activities of the free calcium and phosphate ions during remineralization through the reservoir of bound ACP. The bound ACP, by being in dynamic equilibrium with free calcium and phosphate ions, will maintain the concentrations of the species involved in diffusion into the lesion. Furthermore, dissociation of the CPP - bound ACP will be facilitated by the acid generated during enamel remineralization. This would explain why the CPP – supported metastable calcium phosphate solutions are such efficient remineralizing solutions, since they would consume the acid generated during enamel lesion remineralization by generating more calcium and phosphate ions, including CaHPO_4 , thus maintaining their high concentration gradients.

This study has shown the remineralizing potential of CCP-ACP and Novamin, which make it valuable for the treatment of incipient caries. The study does not take into consideration certain factors within the oral cavity, which may have an influence on tooth surface such as the salivary pH, intake of acidic beverages and foodstuffs. Duration of the application of paste in the present study was limited to three minutes. Previous studies have shown that an increase in application time leads to a proportional increase in the remineralization potential of the tooth [5,30]. These factors might have influenced the final outcome.

Conclusion

Two remineralizing agents which were used in this study i.e calcium sodium phosphosilicate and casein phosphopeptide-amorphous calcium phosphate showed remineralization potential on enamel surfaces. Casein phosphopeptide-amorphous calcium phosphate showed better remineralizing potential than calcium sodium phosphosilicate (Novamin). Hence CPP-ACP can be considered as the materials of choice in remineralizing early enamel carious lesions.

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