Determining the Efficacy of Three Potential Remineralizing Agents on Artificial Carious Lesions

Valluri Pratyusha Sai1, Kakarla Pranitha2, Avula Jogendra Sai Sankar3, Mukthineni Sridhar4, Kundeti Siva Sankar5, Adavikolanu Kesav Ramgopal6

ABSTRACT

Background: Present day, the focus has changed toward increasing the tooth resistance by topical application of remineralizing agents, which has resulted in the remarkable decline in dental caries. However, the remineralizing capacity of these agents remains questionable.

Aim: To determine the efficacy of three commercially available remineralizing agents on artificially created carious lesions on primary teeth.

Materials and methods: A total of 30 sound human primary anterior teeth were selected, decoronated, and randomly divided into three groups of 10 each: group I (FAGamin), group II (Theodent Classic), group III (GC Tooth Mousse). Baseline surface microhardness for each specimen was measured using a Digital Micro Vicker’s hardness tester. Later these specimens were demineralized for 96 hours to assess intermediate surface microhardness values. Further, they were subjected to remineralization with their respective remineralizing agents for 14 days with a pH cycling model and final surface microhardness scores were assessed to determine the extent of remineralization.

Results: The statistical analysis was done using ANOVA and Bonferroni multiple comparison tests, which showed that all the three remineralizing agents exhibited certain amount of remineralization. Group I showed the highest potential compared with the other groups, which was statistically significant (p < 0.05).

Conclusion: All the three groups demonstrated remineralization of carious lesions by virtue of an increase in surface microhardness. Among the tested agents, 38% silver diamine fluoride (SDF) exhibited a superior remineralizing potential.

Keywords: Casein phosphopeptide–amorphous calcium phosphate, Remineralization, Remineralizing agents, Silver diamine fluoride, Theobromine.


INTRODUCTION

“An ounce of prevention is worth a pound of cure,” says an old proverb. Accordingly, application of preventive measures is relevant in the management of many orofacial diseases including that of dental caries, a most prevalent global public health problem affecting numerous urban and rural communities.1,2

Initially, caries dynamics fires up with the drop in the critical salivary pH (5.5) facilitating the growth and proliferation of acidic and acidogenic bacteria, which further plummet the salivary pH leading to enamel demineralization through mineral loss from surface and subsurface enamel leading to a white spot lesion, cavitation, and pulpal pathology in the later stages.2,3

The most effective way to prevent tooth decay is to remineralize it in its most incipient early noncavitated stage. Remineralizing agents deliver the ions needed by creating certain surface coatings that act as diffusion barriers and thereby decrease enamel solubility by the deposition of minerals within the enamel crystallites.4 The World Health Organization expert committee observed a decline in the dental caries prevalence in many countries that was attributed to the widespread use of remineralizing agents.1

Of myriad remineralizing agents invented, fluoride can be considered as the most substantial agent that props up the remineralization to an optimum level.5 Silver diamine fluoride (SDF) is one such fluoride containing a remineralizing agent with a high median lethal dose, which indicates its low toxicity along with the added benefit of silver metal that promotes remineralization.6 The power of cocoa beans with theobromine and milk with casein was also documented to have outstanding remineralization properties.5,7 However, studies comparing the effects of the aforementioned agents on primary teeth are sparse. Thus, to unearth an appropriate novel remineralizing agent that can benefit the oral health in children, the present in vitro study was aimed to assess and compare the remineralizing potential of one novel fluoridated and two nonfluoridated agents when applied to artificial carious lesions on primary teeth.

MATERIALS AND METHODS

After obtaining clearance from the institutional ethical committee, the present in vitro study was planned with a sample size of 30 teeth. The sample included sound human primary caries-free anterior teeth (either extracted for orthodontic reasons, overretained or exfoliated teeth) and were stored in thymol solution until further use. Teeth with dental caries, cracks, white spots, developmental

© The Author(s). 2020 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted use, distribution, and non-commercial reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
defects such as hypoplastic teeth, and discolored teeth were excluded from the study.

The specimens were sectioned at the cervical region with a diamond disc to separate the crown portion of the tooth, which was then embedded in acrylic resin exposing the labial surface. Following sequential polishing with 300, 600, and 1,200 grit waterproof silicon carbide paper, specimens were divided randomly into three groups depending on the remineralizing agent used. Group I: 38% SDF (FAGamin®-Tedequim SRL, Argentina); group II: theobromine as active ingredient (Theodent Classic-TheodentOral Care Products, USA); and group III: casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) as an active ingredient (GC Tooth Mousse—GC America Inc., USA).

The baseline hardness for each specimen was measured by using a Digital Micro Vicker’s hardness tester under an indentation load of 200 g with an indentation time of 20 seconds. Three values were recorded for each sample and their average was taken as the baseline hardness value.

The demineralizing solution was prepared by mixing potassium hydrogen orthophosphate, 0.05 M acetic acid, and 1 M potassium hydroxide to 1,050 mL of distilled water with 2.2 g calcium chloride in it and adjusted to pH 4.5.8

Remineralizing solution was prepared by using 152 mL of distilled water, 0.1665 g calcium chloride, 0.108 g sodium hydrogen phosphate, and 11.25 g potassium chloride with solution pH of 7.8

The specimens were immersed in 500 mL of demineralizing solution for 96 hours to produce artificial carious lesions, following which the surface microhardness was determined. Further, these specimens were subjected to the pH cycling model given by Ten Cate and Duijsters8 for 14 days with their respective remineralizing materials. In the oral cavity, the paste will be quickly diluted by saliva. This effect is simulated by diluting the toothpaste with water in this vitro study.1,9 The chances of solution dilution (group I) will be less as it will be applied on an isolated area.

Following pH cycling, surface microhardness values for each specimen were determined to assess the extent of remineralization. The entire procedure was performed by a single operator. However, to avoid bias, a second operator who was unaware of the prior results evaluated the samples randomly. As the interexaminer variability was not significant \( p < 0.05 \), the scores given by the first investigator were only considered. The data thus obtained were tabulated and subjected to statistical analysis.

**Results**

The mean microhardness, standard deviation, and comparisons were calculated by the paired t test, ANOVA, and Bonferroni multiple comparison tests. The \( p \) value was taken as significant when less than 0.05. The mean surface microhardness of three groups before demineralization ranged between 298.7 KHN and 302.02 KHN with no statistical significance and after demineralization, the values ranged from 244.76 KHN to 257.67 KHN, which were statistically insignificant. After remineralization, the values ranged from 325.88 KHN to 349.25 KHN, which were statistically significant (Table 1). The difference in mean microhardness values was statistically significant in all three groups. However, the difference was more in group I \((149.30 \pm 56.69)\) followed by group II \((88.74 \pm 46.74)\) and III \((68.21 \pm 43.69)\) (Table 1). On intergroup comparison of the difference with Bonferroni multiple comparisons, there was a statistically significant difference when group I is compared with II and III but nonsignificant when group II is compared with III (Table 2).

**Discussion**

Dental caries, a dynamic disease process, transpires by the shift in equilibrium between pathological factors causing demineralization and protective factors causing remineralization. The net demineralization results in the dissolution of the tooth mineral and formation of initial carious lesions, called “white spot” lesions.10,11

The white spot lesion is initial detectable evidence of enamel demineralization in the subsurface region of the tooth characterized by the low calcium and phosphate content. If left untreated, it eventually progresses into frank cavitation.12

Ideally, tooth demineralization at an early stage will be reversed by salivary natural buffer capacity. When the natural systems could not balance the net loss, there are many possibilities to arrest or reverse the progression of the lesion. Remineralization is one such possibility that relies on calcium and phosphate ions assisted by a remineralizing agent that replaces the lost minerals of the tooth and strengthens the lattice network of the tooth structure.7

A variety of newer materials are introduced to promote remineralization like casein phosphopeptides and the ACP complex, sodium calcium phosphosilicate (bioactive glass), xylitol, nanohydroxyapatite, trimetaphosphate ion, alpha-tricalcium phosphate, dicalcium phosphate dihydrate, novamin, enamelol, ion exchange resins, fluoride varnishes, SDF, and theobromine, which were proven to be effective.4,5,13

In the present study, FAGamin (group I), Theodent Classic (group II), and GC Tooth Mousse (group III) were selected as they are commercially available and few studies comparing their efficacy on primary teeth were reported in the literature.

FAGamin, 38% SDF solution, consists of elevated concentrations of silver (253,870 ppm) and fluoride (44,800 ppm) ions. It acts as a powerful cariostatic agent, bactericide with an anti-enzymatic action, protein coagulant, and desensitizer. It exhibits the combined effects of silver and fluoride on the teeth. It has also got its approval by FDA for the treatment of dentinal hypersensitivity.12,14

Theodent Classic was used as a remineralizing agent that has the power of the cocoa bean extract (Theobromine). Theobromine helps harden the tooth enamel, making teeth less susceptible to decay and also found to be a nontoxic alternative to fluoride. The ingredients of this paste are 2–4% concentration of theobromine along with sorbitol, xylitol, glycerin, xanthan gum, sodium benzoate, titanium dioxide, calcium acetate, and sodium hydrogen phosphate.15

The remineralizing technology of CPP–ACP in GC Tooth Mousse is based on phosphopeptide from milk protein, casein. It contains purified water, glycerol, CPP–ACP, D-sorbitol, silicon dioxide, CMC-Na, propylene glycol, titanium dioxide, xylitol, phosphoric acid, guar gum, zinc oxide, and sodium saccharin.16

The pH cycling model is a valuable tool to determine the efficacy of materials under laboratory conditions.17 The pH cycling protocol of Ten Cate and Duijsters is of particular interest because it simulates in vivo high caries risk conditions.1

Microhardness changes measurement was selected for this study as it is appropriate for the enamel with a fine nonhomogeneous microstructure prone for cracking. This can be done by using either a Vicker’s microhardness tester, scanning
Determining the Efficacy of Three Potential Remineralizing Agents on Artificial Carious Lesions

Electron microscopy (SEM), environmental SEM, or the Diagnodent method. A Vicker’s microhardness tester was used in the study for the precise evaluation of the mineral changes. Gutierrez and Reyes also advocated the use of a Vicker’s indenter in the tooth hardness studies for precise measurements.

It was observed that mean surface microhardness of three groups before demineralization (baseline) had no statistical significance (p > 0.05) indicating that all the specimens had no major variations in their surface topography. But the demineralized specimens surface hardness was reduced in all the groups owing to mineral loss and there was no statistical significance (p > 0.05) between the groups, which implies that all the specimens were demineralized to a similar extent.

A significant (p < 0.05) increase in mean surface hardness values was noticed after remineralization through pH cycling, which could be attributed to the deposition of the inorganic contents which in turn elicit their remineralizing potential.

Among the test groups, group I exhibited a higher remineralization potential followed by group II and group III. On comparison, statistical significance was observed when group I is compared with group II and III and nonsignificance between groups II and III indicating the potency of SDF. This superior effect of SDF might be due to its antibacterial effect that can prevent biofilm formation, unique matrix metalloproteinases (MMPs) inhibition, enzyme inhibition, and dentinal tubule occlusion properties.

When the cariostatic effect of SDF has been observed, it was found that silver ions are bactericidal, which interfere with the synthesis of cellular polysaccharides and colonization. Fluoride ions influence enzymes, such as enolase and proton-extruding adenosine triphosphatase (ATPase), which interfere with the bacterial carbohydrate metabolism. Silver diamine fluoride inhibits MMPs and this protects tooth collagen from degradation. An insoluble protective layer of calcium fluoride, silver phosphate, and silver protein that forms after application decreases the calcium and phosphorus loss from the carious lesions by occluding the tubule orifices and fluorohydroxyapatite formed arrests caries.

Likewise, Mei et al. evaluated the effect of 38% SDF on cariogenic biofilms and dentinal carious lesions and the results revealed lesser bacterial growth and a higher percentage of calcium and phosphorus deposition. Remineralization efficacy of SDF was further confirmed by Soekanto et al. along with nanosilver fluoride (NSF) and propolis fluoride (PPF) and depicted the precipitates of apatite compounds, calcium compounds, and metal compounds in the treatment groups.

Chu et al. suggested that SDF is found to be more effective on dentinal carious lesions. The reason is that the dentinal tissue has a higher content of protein, carbonate, and phosphate available to react with silver. In contrast, these compounds are scarce in the enamel tissue. Based on this inference, its effect could be more on the primary teeth compared to permanent teeth as the primary teeth contain more organic content.

Similarly, Mohammadi and Far compared the effect of fluoridated varnish and SDF solution on primary teeth enamel resistance to demineralization through a pH cycling study. Silver diamine fluoride exhibited a superior resistance compared to fluoridated varnish and the hypothesis behind it could be due to its silver content (253,870 ppm), which has an intense antibacterial effect on the cariogenic biofilm that hinders caries progression and fluoride (44,800 ppm) that has the ability to increase enamel surface microhardness and reduce enamel surface mineral loss.

Further, the present study results exhibited a significant increase in the mean microhardness values in group II, which were greater when compared with group III but not statistically significant. The increase in surface microhardness could be due to the interstitial substitution of theobromine ions on apatite crystal, which increases the density and consecutively the surface microhardness.

When the effect of theobromine on the enamel has been elucidated, it has been found that the ions in theobromine (C7H8N4O2) have a smaller diameter (152–170 pm) than that of enamel hydroxyapatite crystal microtunnels (176 pm). So on application, smaller ions of theobromine can pass the tunnel and generate an interstitial reaction with apatite crystals. This increases the density of the apatite crystal and in turn the enamel surface hardness.

Amaechi et al. investigated the remineralization potential of theobromine and standard NaF dentifrice through pH cycling and found that theobromine in an apatite-forming medium can enhance the remineralization potential. Similarly, Nasution et al. analyzed the difference in hardness between fluoride and theobromine application and concluded that both are equally able to increase the hardness of the enamel surface. This was further confirmed by Amaechi et al. who concluded that theobromine-containing toothpastes with and without fluoride have equal potential in occluding the dentin tubules. Irawan et al. evaluated the effect of 200 mg/L theobromine gel on enamel microhardness and concluded that 200 mg/L theobromine gel can increase the enamel surface hardness.

Even though a significant increase in the mean microhardness values was observed after remineralization in group III, this increase was less compared to the groups I and II. The reason for increased

---

**Table 1: Mean surface microhardness values**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline (KHN)</th>
<th>Intermediate (KHN)</th>
<th>Final (KHN)</th>
<th>Difference between intermediate–final values</th>
<th>p value of the difference (paired t test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>300.58 ± 27.58</td>
<td>244.76 ± 25.28</td>
<td>394.25 ± 47.66 *</td>
<td>149.30 ± 56.69</td>
<td>0.000*</td>
</tr>
<tr>
<td>II</td>
<td>297.8 ± 21.92</td>
<td>255.12 ± 27.41</td>
<td>343.95 ± 38.84 *</td>
<td>88.74 ± 46.74</td>
<td>0.000*</td>
</tr>
<tr>
<td>III</td>
<td>302.02 ± 20.00</td>
<td>257.67 ± 22.83</td>
<td>325.88 ± 44.87 *</td>
<td>68.21 ± 43.69</td>
<td>0.001*</td>
</tr>
<tr>
<td>p value (ANOVO)</td>
<td>0.851</td>
<td>0.490</td>
<td>0.005*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2: Intergroup comparison of the mean microhardness values**

<table>
<thead>
<tr>
<th>Groups</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I–II</td>
<td>0.049*</td>
</tr>
<tr>
<td>I–III</td>
<td>0.005*</td>
</tr>
<tr>
<td>II–III</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

**Statistical analysis:** one-way ANOVA and paired t-test

* denotes statistically significant if p < 0.05
microhardness values could be attributed to the presence of CPP that stabilizes and localizes calcium phosphate to form stable apatitic products.

Casein phosphopeptides in CPP–ACP contain multiphosphoseryl sequences, which when applied on the enamel can stabilize and localize ACP at the tooth surface, thereby increasing the level of calcium and phosphate in plaque that acts as a calcium phosphate reservoir. This supersaturation with respect to the tooth enamel leads to the formation of stable crystalline phases, such as octacalcium phosphate or apatitic products, which decreases enamel demineralization and enhances enamel remineralization.16,28

The present study results were in accordance with the study outcome by Balakrishnan et al.7 who stated that CPP–ACP has better remineralization potential on comparing the microhardness changes produced by 0.21% sodium fluoride with f-TCP (Clinpro) and bioactive glass Novamin (SHY-NM) containing dentifrices. However, the insoluble silver metallic phase formation in group I and the dentinal tubule occlusion in group II could have contributed to superior results than group III.

Researchers have recommended the addition of certain components like fluoride to improve the efficacy of CPP–ACP. This was confirmed by Shetty et al.29 who assessed the efficacy of three remineralizing agents CPP–ACP (GC Tooth Mousse), CPP–ACPF (GC Tooth Mousse Plus), and NaF (Phos-Flur) by analyzing the surface microhardness changes. Remineralization efficacies are in the order of CPP–ACPF followed by NaF and CPP–ACP. Similarly, Patil et al.2 conducted an in vitro study to evaluate the remineralizing potential of CPP–ACP, CPP–ACPF, and tricalcium phosphate fluoride (TCP-F) on an artificial enamel carious lesion. Tricalcium phosphate fluoride exhibited a superior efficacy followed by CPP–ACPF and CPP–ACP. Mendes et al.30 through their 90-day trial observed that CPP–ACPF exhibited superior remineralization followed by CPP–ACP and fluoride gel and concluded that the use of CPP–ACP is a good alternative for the remineralization of white-spot lesions.

Based on the present study results, it can be suggested that an appropriate remineralizing agent can resist the early lesion progression. Remineralizing agents should be prescribed based on lesion’s size, site, caries activity, and other potential risk factors, which can save the resources in terms of time, money, and manpower by utilizing the tool of proactive intervention.

However, certain limitations exist in this in vitro study like the pH cycling model could not entirely simulate the oral conditions as it depends on individuals’ eating habit, oral hygiene practice, fluoride usage, composition and quality of saliva, and plaque. Apart from this, the study also focused on the quantitative changes. However, to achieve more conclusive results, qualitative changes like the enamel crystal formation pattern and its chemical structure and acid solubility must be assessed using advanced quality assessment techniques.

Silver diamine fluoride could be promising in managing dental caries in high-risk groups. However, black staining following SDF application may influence the acceptability of the treatment. Recently, Patel et al.31 suggested the use of potassium iodide (KI) immediately after SDF application, which could reduce the noticeable staining of the carious dentine or the surrounding enamel. So, future research can focus on the elimination of the drawback of SDF without any reduction in its cariostatic activity and also on the evaluation of the difference in the cariostatic activity on the primary and permanent teeth enamel.

CONCLUSION

The following conclusions were drawn from the present study:

- All the three groups demonstrated the remineralization of artificial carious lesions by virtue of an increase in surface microhardness values.
- Silver diamine fluoride exhibited superior remineralization potential followed by theobromine and CPP–ACP.
- Silver diamine fluoride is a cariostatic agent used to adjunctively manage dental caries in high-risk groups. However, theobromine or CPP–ACP can be opted for the individuals with heavy metal allergies and ulcerative gingivostomatitis.
- Based on individuals potential risk factors, an appropriate remineralizing agent should be selected which could alter the caries balance, especially in individuals with a high cariogenic bacterial challenge.

REFERENCES


