“War of the Varnishes – A Comparative Evaluation between Three Remineralizing Agents Using Confocal Microscopy”

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Abstract

Objective: Dental caries is still considered even in this day and age as the major contributor to the vast multifarious oral disease conditions affecting people all around the globe. Once thought to be irreversible, dental caries lesions are now being approached with the focus on remineralization of the tooth structure by means of artificially designed remineralizing agents. Fluoride was considered the mainstay for the remineralization regimen, but today numerous manufacturers have opened up the options with a wide selection of remineralizing products ranging from dentifrices to varnishes. The present in vitro study compares three such options for their efficacy in remineralizing an artificially produced carious lesion in permanent human teeth. The aim of the study was to compare and evaluate the remineralization potential of Copal Care varnish (sodium fluoride), MI varnish (Casein phosphopeptide-amorphous calcium phosphate [CPP-ACP-F]), and Cervitec F+ (chlorhexidine varnish) using confocal microscopy.

Materials and Methods: Enamel windows were created on the middle third of the buccal surface of the crown of orthodontically extracted permanent premolars. The samples were demineralized, sectioned using a hard tissue microtome and randomly divided into three experimental groups of 10 each. Group A specimens were coated with Copal Care varnish, Group B with MI varnish, and Group C with Cervitec F+ varnish using applicators for 60 s. The samples were then subjected to a pH cycling for a period of 5 days, where the samples underwent cyclic demineralization and remineralization within the respective artificially prepared solutions. The samples after pH cycling were subjected to confocal scanning microscopic analysis and the collected data were tabulated and statistically analyzed using SPSS software (version 27). The data were subjected to Fisher’s paired t-test and one-way ANOVA.

Results: All the three groups showed significant remineralization of the artificial carious lesion on the confocal images. Copal Care showed the highest remineralization followed by Cervitec F+ and MI varnish. The differences in mean lesion depth values among three groups at baseline and post-treatment of all three products were found to be not statistically significant with the P ≤ 0.05. However, the differences in mean lesion depth between the three groups were statistically significant with P ≥ 0.05.

Conclusion: Copal Care showed the greatest remineralization potential followed by Cervitec F+ and MI varnish, respectively.

Key words: Chlorhexidine varnish, Confocal microscopy, MI varnish, Sodium fluoride varnish

INTRODUCTION

Dental caries hails even in this era of advancements as the leading cause of oral health disease that affects millions worldwide, causing substantial loss in the productive hours which amounts to significant number each year. This oral condition takes several forms and varies in intensity and frequency among individuals affecting the more industrialized population by observed trend.[3]

The concept of cariogenicity is based on two episodic processes taking place one after the other – demineralization and remineralization.[3] The two key processes rely on 4 important factors – a susceptible host, a colony of bacteria, presence of simple carbohydrates, or sugars in the diet and adequate time for the bacteria to be in contact with the sugars and to the dental hard tissues to form soluble acids mainly lactic acid.[3]
The first sign of demineralization is the appearance of white spot lesions indicating a subsurface demineralization which if left uncontrolled by use of different remineralizing agents leads to cavitation. The condition is of particular concern in patients undergoing orthodontic treatment, where due to lack of proper cleansing there is a high risk for incipient lesion.[4] The major concern is the ignorance of these lesions in the beginning and use of proper protective agents, as most remain subclinical. For years fluoride has been considered the gold standard form the treatment of incipient lesions in the enamel since its development in the 1970s. The natural earth mineral is extracted and used in a surfeit range of dental care products ranging from dentifrices to varnishes.

The products all have one function, to elevate the fluoride concentration locally to strengthen the enamel structure by formation of fluoridated hydroxyapatite crystals that have an exceptional capacity to oppose demineralization when compared to naturally formed hydroxyapatite crystals. It begins to remineralize the incipient carious lesions in the presence of calcium and phosphate.[5]

Among the vast selection of fluoride products varnishes has shown the best remineralizing potential because of its better and prolonged adhesive action which increases the fluoride concentration for maximum formation of fluorapatite. Over the years, fluoride varnishes have been in supply in different concentrations of fluoride ranging from 7000 ppm to 26,000 ppm. Many studies have been conducted to study the efficacy of these various concentrations of fluoride for remineralization of enamel. In recent years, varnishes have been modified using various natural and chemical substitutes both to try increase efficacy of the product and to overcome the side effects of fluoride use at excessive concentrations.[6]

Sodium fluoride has been recognized as an efficient remineralizing agent with an effective fluoride concentration of 23,600 ppm, more than any other fluoride combinations. It interacts with the oral fluids, combines with calcium and phosphate saturated within the oral fluids, and forms fluorapatite. The efficiency of the product depends on the surface concentration of the varnish and the frequency of application.[7]

Casein phosphopeptide-amorphous calcium phosphate (CPP ACP) was introduced in the year 1998, CPP-ACP comprises nanocomplexes of milk protein CPP with ACP. It promotes remineralization of the carious lesions by maintaining a supersaturated state of essential minerals and at the same time it also hinders colonization of dental surfaces by cariogenic bacteria.[8]

Cervitec is a varnish designed for mechanical protection of the tooth structure, fluoridation, and antimicrobial action. It contains 1400 ppm fluoride from ammonium fluoride in a varnish base with ethanol and water as solvents with the additional action of 0.3% chlorhexidine (CHX).[9] The fluoride content prevents enamel demineralization, promotes re-mineralization, and exerts minimal anti-plaque action. The CHX provides the major anti-microbial cover especially against Streptococcus mutans, bringing complete overall protection. CHX being cationic has both hydrophilic and hydrophobic properties which help it bind to the negatively charged S. mutans cell wall especially in higher concentration, leading to disruption in the integrity of the cell wall, leaking of intracellular contents and finally bacterial cell death.[10]

The lack of literature on the remineralization potential and antimicrobial action of Cervitec F against S. mutans and a comparative evaluation between the alternatives, two widely accepted products (copal care and MI) has paved way to the present study which is a comparative evaluation between Copal Care varnish (sodium fluoride), MI varnish (cpp acp-f), and Cervitec f+ CHX varnish in remineralization of samples of enamel sections in *in vitro* and evaluation and visualization of remineralization through confocal microscopy.

**MATERIALS AND METHODS**

The following *in-vitro* study was conducted in the department of public health dentistry, Asan Dental College and Hospital, Chennai, India, for a period of 30 days after an ethical approval was obtained from the Institutional review board.

**Sample Selection and Preparation**

The sample size for the study was determined at \( n = 30 \) which provided a maximum of 10 samples per experimental group. Orthodontically extracted sound permanent premolars were collected in which there were no signs of caries, attrition, hypoplasia, discoloration, or any other developmental defects. The samples were put through these inclusion criteria to provide a comparatively equal rate of progression of demineralization in each sample. The selected sample was cleansed of debris, stains, and calculus and stored in 10% formalin solution.

The selected samples were then prepared for enamel window preparation as shown in Figure 1. The enamel window provided a controlled area of demineralization. It was created by marking a 3×3 mm square on the middle third of the buccal surface of each premolar. The remainder of the tooth was covered in acid resistant nail polish and allowed to dry before beginning the pH cycling procedure.
Preparation of Demineralizing and Remineralizing Solution

The incipient carious lesions were created on the enamel window of the samples by placing them in an artificially prepared demineralizing solution as shown in Figure 2. The demineralizing solution contained a blend of calcium chloride (2.0 mmol/L), tri sodium phosphate (2.0 mmol/L) in acetate buffer (75 mmol/L) solution at pH 4.4 as shown in Figure 3, that is, at the critical pH at which there is pronounced demineralization of hydroxyapatite crystals.[11] The samples were suspended inside test tubes with measured, equal quantity (10 ml) of the prepared demineralization solution for a period of 4 days at room temperature(37°).

A remineralizing solution was also prepared to place the samples which were treated with the respected products in the artificial media to observe the alterations in baseline lesion depth of the demineralized enamel sections after a pH cycling of 7 days. The remineralizing solution contained NaPO₄ - 3.90 mM, NaCl - 4.29 mM, KCl - 17.98 mM, CaCl₂ - 1.10 mM, MgCl₂ - 0.08 mM, HSO₄ - 0.50 mM, NaHCO₃ - 3.27 mM, distilled water, with pH maintained at 7.2.[12]

Experimental Groups

The demineralized samples were randomly divided into 3 groups with 10 samples each:
- Group A (n = 10) - Copal Care (sodium fluoride)
- Group B (n = 10) - MI Varnish (CPP-ACP-F)
- Group C (n = 10) - Cervitec F+ (CHX varnish)

Sectioning of Samples

The samples of each group were mounted in wax blocks [Figure 4], sectioned using a hard tissue microtome (Leica SP 1600) [Figure 5] into longitudinal sections of thickness 150–200 µ [Figure 6]. The samples were then mounted on to glass slides. The sections were all stained with rhodamine.

B solution and the baseline lesion depth for each sample were determined using confocal laser scanning microscopy.

Application of Remineralizing Products and pH Cycling

The specimens in group A were coated with Copal Care [Figure 7] using applicators for 60 s per specimen. The procedure was repeated for the application of MI varnish [Figure 8] on the specimens of group B and Cervitec+F [Figure 9] on specimens of group C. Once dry the samples were then subjected to a pH cycle of alternative demineralization and remineralization. The demineralization of the samples was carried out in the prepared demineralizing solution for 3 hours. The samples were then transferred into the remineralizing solution, where they underwent remineralization for a period of 21 hours. The consecutive cycle was followed for a period of 7 days.

Post-treatment Analysis

The samples after a period of 5 days of pH cycling were stained with freshly prepared Rhodamine B solution for 1 h and mounted on frosted glass slides. The specialized stain integrates into the demineralized structures within the samples and provides contrast from the sound tooth structure. The samples were washed thoroughly with phosphate solution to remove excess stains and were remounted with 80% glycerol mountant. Cover slips were placed on top making sure of no air entrapment and edges of the coverslips were coated with transparent nail enamel to prepare the slides for confocal analysis. A confocal laser scanning microscope (Leica TCS SL inverted microscope) was used to measure the post-treatment lesion depth. The software analyzed the linear depth of fluorescence and also the average or total fluorescence. The images were captured from the buccal surface that is one each from either side of the mid-point measured from the occluso-cervical length of the tooth at (×5) magnification and for excitation and emission range of 498–514 nm wavelength an, Argon laser was used at 488 nm wavelength. Two images were captured from either side of the midpoint of occluso-cervical length on the buccal surface.

Statistical Analysis

The values for each specimen were noted and tabulated. A statistical analysis was done using SPSS software (version 27). The data were subjected to Fisher’s paired t-test and one-way ANOVA.

RESULTS

The three groups showed significant remineralization of the artificial carious lesion based on the confocal images although, Copal Care showed the highest remineralization [Figure 10] followed by Cervitec F+ [Figure 11] and MI varnish [Figure 12]. Table 1 depicts the mean lesion depth values among three groups at baseline. The mean lesion depth value was found to be higher for Group A (576.26 ± 1.17) closely followed by Group B (546.56 ± 1.19) and then Group C (422 ± 1.24). The difference in lesion depth was found to be significant statistically. Table 2 depicts the mean lesion depth values among the three groups after pH-cycling. The mean lesion depth value was found to be higher for Group A (383 ± 1.15) followed by Group B (365.61 ± 1.43) and then Group C (234.10 ± 1.10). The difference in lesion depth was found to be significant statistically.

DISCUSSION

Incipient carious lesion is the “white spots” found on the enamel subsurface, formed by decalcification of the enamel. The surface of the enamel is intact with no
cavitation. It is the initial step in the process that leads to the clinically significant caries associated cavities, sensitivity, and pain. This stage of caries is often left unnoticed due to the shortfall of conventional signs and symptoms of a carious lesion. As discussed earlier, the formation of these lesions are multifactorial. The initial step in the caries formation is the plaque formation. The primary organism that initiates colonization on the enamel surface is the well-known, S. mutans. The organism plays the key role beginning from the accumulation of plaque, acid formation, and finally the decalcification of enamel.

Demineralization begins as the acids produced by breakdown of sugars within the plaque bacteria reduce the oral pH. The lowered pH un-saturates the plaque fluids of calcium and phosphate ions. At this point, the calcium and phosphates from the enamel leaches out until the plaque fluids are saturated again. Demineralization controls the progression of caries. Remineralization on the other hand causes reversal of the ion transfer and deposits calcium and phosphates until the lesion is saturated. It occurs only as the pH rises above the critical pH.

It has been reported that even trace quantities of fluoride ions are effective in formation of hydroxyapatite crystals. Hence, fluoride has been the key ingredient within most

| Table 1: Mean lesion depth values among three groups at baseline |
|-----------------|-----------------|-----------------|-----------------|
| Groups          | Lesion depth (Mean±SD) | F-value         | Degrees of freedom | P-value |
| Group A (sodium fluoride) | 576.26±1.17     | 46023.013       | 2                | 0.000*  |
| Group B (MI)    | 546.56±1.19     |                 |                  |         |
| Group C (Cervitec F) | 422.24±1.24   |                 |                  |         |

*One-way ANOVA. The difference in lesion depth was found to be significant statistically.

| Table 2: Mean lesion depth among three groups after pH-cycling |
|-----------------|-----------------|-----------------|-----------------|
| Groups          | Lesion depth (Mean±SD) | F-value         | Degrees of freedom | P-value |
| Group A (sodium fluoride) | 383±1.15       | 43226.633       | 2                | 0.000*  |
| Group B (MI)    | 367.61±1.43     |                 |                  |         |
| Group C (Cervitec F) | 234.10±1.10   |                 |                  |         |

*One-way ANOVA. The difference in lesion depth was found to be significant statistically.

The conventional treatment modalities would involve the removal of the plaque and calculus, extension of cavities to sound enamel with proper outline form, and sealing of those cavities with either dental cements or sealants. The treatment provides relief but is not conservative of the tooth structure. The early clinical detection of the incipient lesions and their objective monitoring would help remineralize the lesion to sound form without the need to cut enamel or dentin.

Figure 1: Preparation of enamel window
Figure 2: Specimens placed in demineralizing solution
Figure 3: pH meter
enamel remineralization products. Fluoride varnishes were developed in the late 1960s and 70's. Fluorides showed topical anti-cariogenic effects which were ascribed to the reduced solubility of fluoridated crystal lattice of enamel or also known as fluorapatite.\(^{[18]}\) Even though rare the fluoride varnishes due to the high fluoride content (22,600 ppm) does have side effects, majorly fluorosis, and fluoride toxicity.\(^{[19]}\)
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The present study utilizes MI varnish. Even though conventional CPP shows efficient remineralization, because of the added benefit of fluoride (NaF 0.2%), CPP-ACPF shows marginally more amount of remineralization than CPP-ACP. Cervitec+F is a relatively new product which other than the anti-cariogenic action of fluoride, incorporates the antimicrobial action of CHX against plaque bacteria especially S. mutans, the primary colonizer. CHX has been reported effective in the control of plaque formation and caries prevention. 0.2–2% of CHX is effective enough to reduce the self-degradation of collagen fibrils by inhibiting host-derived protease activity in demineralized dentin and influence dentin remineralization. The combination of CHX and fluoride in form of a varnish has shown similar effectiveness in the prevention of caries in both permanent and primary dentitions. Even though proven efficient there is a lack of literature to substantiate the efficacy of CHX varnish compared to the fluoride and CPP-ACP alternatives.

A confocal scanning microscopic analysis of the demineralized enamel sections provided substantial information on the difference between the baseline lesion depth and post-treatment lesion depth between the groups. Confocal microscopy provided the control of field depth, elimination of background information from the focal plane and collection of serial optical sections from the thick enamel sections. The expense, chances of artefacts, and the difficulty to mount thick sections into the scanning electron microscope chamber made controlled low strength materials more feasible for the present study.

The post-treatment results showed that the specimens applied with sodium fluoride showed the greatest remineralization of the incipient carious lesions when compared to Group B and Group C, i.e., CPP-ACPF and CHX varnish, respectively. The difference was found to be statistically significant. This was in accordance with the study by Chokshi et al., which concluded that sodium fluoride does have better efficacy in remineralization of in-vitro produced incipient enamel lesions when compared to CPP-ACP within the time intervals of 20 days and at 40 days. Contrary to the results of the present study in a study conducted by Akin et al., which compared the efficacy of two mouth washes containing sodium fluoride and casein phosphopeptide, respectively, in treatment of white spot lesions in 80 patients, post-orthodontic therapy, the group which used CPP-ACP showed higher remineralization of the white spots within the time frame of 6 months.

Now in the present when comparing the fluoride group (Group A) and CHX group (Group C) both lesions show

CPP is milk protein derived peptides which substantially increase the levels of calcium phosphate in plaque which in turn decreases enamel demineralization.
significant reduction in baseline lesion depth although group A had better results. Similar results were found to be in a study by Naidu et al., where in an in vitro study conducted in children the groups containing fluoride, CHX, and a combination of both had increased post-treatment levels of enamel calcium and phosphate as compared to the negative control group. A randomized control study conducted by Papas et al. too concludes that there is significant reduction in the number of un-cavitated carious lesions when treated with CHX varnish in an adult population over a period of 6 months. There is currently scarce literature testing the effectiveness of CHX varnish in an in vitro scenario and a comparison between CHX and CPP-ACP varnishes.

A study by Somasundaram et al. comparing effect of paste containing CPP-ACP, fluoride, on enamel remineralization, it was concluded that enamel surfaces treated with the CPP-ACP paste exhibited the least lesion depths followed by the enamel surfaces treated with the fluoride toothpaste and control group, respectively. A similar study conducted by Datta et al., on 45 subjects with occlusal white spot lesions, groups that were treated with CPP-ACP showed superior remineralization that the fluoride treated group. The results from the following studies are contrary to the results obtained in the present study. However, in every study, the results of remineralization by fluoride and CPP-ACP have no significant variations.

Limitations

The in vitro study does not account for the dynamic microbiological system in the oral cavity. This glaring constraint must be considered and assumptions must be made with caution when compared to clinical studies. Furthermore, clinical trials are required to validate the findings in the current study.

CONCLUSION

Copal Care showed the greatest remineralization potential followed by Cervitec F+ and MI varnish, respectively. The differences in mean lesion depth values among three groups at baseline and post-treatment of all three products were not statistically significant with the \( P \leq 0.05 \). However, the difference in mean lesion depth between the three groups was statistically significant with \( P \geq 0.05 \). Fluoride does have a profound effect on the level of caries progression but we cannot always recommend high fluoride strategies. They cannot be followed due to the adverse effects of fluoride topically and systemically. Hence, there is still a need for remineralizing agents with less fluoride content and comparable antacaries progression properties to the current high fluoride options.

REFERENCES


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