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Comparative evaluation of application of different fluoride varnishes on artificial early enamel lesion: An *in vitro* study

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Abstract

India

Introduction: In an attempt to manage noncavitated carious lesions noninvasively through remineralization, a range of novel fluoride varnishes with additional remineralizing agents have been made available for clinical application. Aim and Objectives: The aim of this study was to compare and evaluate the remineralization potential of three commercially available varnishes on artificial enamel lesions. Materials and Methods: This *in vitro* study involves eighty intact enamel specimens prepared from premolars extracted for orthodontic purposes. After specimen preparation, the eighty samples were divided randomly into two groups (n = 40) for measurement of baseline surface Vickers microhardness and baseline calcium/phosphorus ratio (% weight) through EDAX testing. Thereafter, the specimens were subjected to demineralization for 96 h to induce initial enamel lesions and the measurements were repeated. Following demineralization, each of the two groups was divided randomly into four subgroups (n = 10) for which one was used as the control group and the others three were allotted to each of the three test varnishes. After varnish application, all the specimens were subjected to a pH cycling regimen that included alternative demineralization (3 h) and remineralization (21 h) daily, for 5 consecutive days. The Vickers microhardness and EDAX measurements were then repeated. Results: One-way ANOVA and *post hoc* Tukey«SQ»s tests were conducted for multiple group comparison. All the three commercially available varnishes were capable of remineralizing initial enamel lesions that were induced artificially. No difference was noted in the remineralizing efficacy of the varnishes despite their different compositions. MI VarnishTM (casein phosphopeptide-amorphous calcium phosphate fluoride varnish) showed slightly better recovery in surface microhardness as compared to the other two varnishes. Conclusion: All the varnishes used in this *in vitro* study are capable of reversing early enamel lesions.

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Full Text

Dental caries is one of the main causes of tooth loss for all human beings across age and gender. The progression of the early enamel lesion is a slow process, and this early carious lesion is reversible via the process of remineralization, whereby calcium and phosphate ions are supplied from a source external to the tooth to promote ion deposition into crystal voids in demineralized enamel to produce net mineral gain. [1],[2] This principle is the key to "Preventive Dentistry," wherein "Extension for Prevention" has given way to the new paradigm of "Minimally Invasive Dentistry." In the past two decades, caries research has been focused on the development of methodologies for remineralization of carious lesions. [3] In 1964, Schmidt presented a method of applying NaF in a natural colophony base, which could adhere to tooth surfaces in the presence of saliva. [4] This led to the production of fluoride varnishes. The varnishes were originally developed to prolong the contact time between fluoride and tooth enamel. Since the advent of the first varnishes, researchers have strived to improve both efficacy and delivery of fluorides in varnishes. There is a sound rationale for the addition of calcium ions to fluoride varnishes has not been studied widely. Hence, the purpose of this study was to find out the effect of addition of agents such as amorphous calcium phosphate (ACP) and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) to fluoride varnishes by comparing their remineralization potential.

Aim and objectives

To study the remineralization potential of three commercially available varnishes on artificial enamel lesions. To evaluate quantitatively the differences in the remineralization potential of

MI Varnish[™] (5% sodium fluoride varnish with Recaldent[™] (CPP-ACP) Technology, GC Corporation, Tokyo, Japan)Premier ® Enamel Pro ® Varnish (5% sodium fluoride varnish with ACP Formula, Premier Dental Products Co., Canada) and Flor-Opal ® Varnish White (5% sodium fluoride varnish, Ultradent Products, Inc., USA). To compare the recovery in surface microhardness after the application of the three varnishes.

MATERIALS AND METHODS

For the purpose of this in vitro study, a total of eighty enamel specimens were prepared from forty healthy extracted premolars. Sound, healthy noncarious premolars extracted for orthodontic purpose with all surfaces intact were selected, whereas any tooth with visible cracks, hypoplasia, white spot lesion, or caries on any surface was excluded from the study. After thorough scaling with an ultrasonic scaler and polishing with a prophylaxis paste and a rubber cup, the radicular part of each of the forty teeth was removed. The coronal part of each tooth was longitudinally sectioned in a mesiodistal direction into two sections using a high-speed diamond tipped disc, and in this way, two enamel specimens were prepared from each tooth giving a total of eighty enamel specimens. After this, the eighty specimens were randomly divided into two groups of forty specimens each.

Group 1: Microhardness testing (n = 40)Group 2: Scanning electron microscopy with an energy dispersive X-ray analysis attachment (SEM-EDAX) testing (n = 40). The first group of forty specimens allotted to microhardness testing was mounted in acrylic blocks to stabilize the enamel specimens. For this, custom-made rectangular aluminum molds of size 3 cm × 2 cm were used, and self-cured acrylic resin was poured into it. The enamel specimens were embedded on top of the partially set acrylic and allowed to set. After this, an acid-resistant nail varnish (Avon Products, India) was applied around the exposed enamel surface of all the specimens leaving a window of 3 mm × 3 mm of enamel exposed at the center.

The second group of the forty sectioned specimens allotted to EDAX testing was not mounted and were used as it is. An acid-resistant nail varnish (Avon Products, India) was applied on the intact enamel surface of all the specimens leaving a window of 3 mm × 3 mm of enamel exposed at the center.

The forty specimens from Group 1 were tested for initial surface microhardness using the digital Vickers microhardness tester (Model: HMV-2T; Shimadzu Co. Ltd.), and the other forty

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from Group 2 were subjected to EDAX (Nova Nano SEM 450; FEI Ltd.) to determine the baseline calcium and phosphorus (Ca and P) and values (% weight). The objective of baseline microhardness and Ca/P content determination is to compare and calculate the changes that occur after induction of enamel lesions and after pH cycling.

All the prepared specimens of Groups 1 and 2 were immersed in individual plastic containers containing 10 ml of demineralization solution each, for 96 h, in an incubator at a temperature of 37°C. [5] The buffered demineralizing solution [5] was prepared using analytical grade chemicals and double distilled water, and it contained

2.2 mM calcium chloride2.2 mM sodium phosphate0.05 M acetic acid. The pH of the solution was checked using a digital pH meter (Systronics pH system 361) and was adjusted to 4.2 using 1 M potassium hydroxide. The solution was freshly prepared and changed daily to avoid supersaturation.

After induction of enamel lesions, the surface microhardness test for Group 1 and SEM-EDAX test for Group 2 were repeated to obtain the postdemineralization values.

Each of the two groups was then further divided randomly into four subgroups of ten specimens each based on the varnish to be applied.

Group A: No varnish (control Group)Group B: Flor-Opal ® Varnish White groupGroup C: Premier ® Enamel Pro ® Varnish groupGroup D: MI Varnish™ group. After the demineralization cycle, the specimens were thoroughly cleaned with double distilled water and patted dry before application of the varnish. The varnish was applied with the help of the applicator tips provided by the manufacturer.

The experimental process attempted to imitate the changes in pH in the oral environment for which the treated specimens were subjected to a pH cycling regimen that included alternative demineralization (3 h) and remineralization (21 h) daily, for 5 consecutive days. [5]

For the demineralization phase, the demineralization solution used for the induction of enamel lesions was used, and for the remineralization phase, a synthetic remineralizing solution was prepared. The inorganic composition of the remineralizing solution [5] is similar to that of natural saliva. It consisted of:

1.5 mM calcium chloride0.9 mM sodium phosphate0.15 M potassium chloride. The remineralizing solution was prepared using analytical grade chemicals and double distilled water. Its pH was measured using a digital pH meter (Systronics pH system 361) and set to 7.0. The specimens were rinsed with double distilled water before changing the solutions. Both the solutions were freshly prepared and changed daily. After pH cycling, the surface microhardness for Group 1 (A-D) and the SEM-EDAX measurements for Group 2 (A-D) were repeated.

RESULTS

The surface microhardness of the enamel specimens was measured using a digital Vickers microhardness tester (Model: HMV-2T; Shimadzu Co. Ltd.) and expressed in terms of Vickers microhardness number (VHN). The values obtained were then subjected to statistical analysis.

The microhardness values within the same group at different treatment stages were compared using the Student's paired t-test, whereas comparison between the various treatment groups was done using one-way ANOVA [Table 1]. This was followed by post hoc analysis as each group contained three subgroups based on the stage of treatment [Table 2]. p < 0.05 was considered statistically significant.{Table 1}{Table 2}

The percentage of microhardness recovery for each of the treatment groups was calculated using the following formula: [6]

[INLINE:2]

The Ca and P analysis was done using SEM-EDAX (Nova Nano SEM 450; FEI Ltd.) and expressed in terms of atomic weight %. Using these values, the Ca/P ratios were analyzed for each sample.

The values within the same group at different treatment stages were compared using the Student's paired t-test for which P < 0.05 was considered statistically significant. The comparison of the Ca, P, and Ca/P values between the four groups was done using the one-way ANOVA test followed by post hoc Tukey's honestly significant difference analysis [Table 3] and [Table 4].{Table 3}{Table 4}

DISCUSSION

Early diagnosis of incipient lesions has led to a new era in "Preventive Dentistry." The efficacy of fluoride in caries prevention has been well documented since the 1930s. Over the past few years, topical fluoride agents appear to have dominated in preventing caries development. [7] Fluoride-containing varnishes were developed during the late 1960s and early 1970s in an effort to improve the shortcomings of existing topical fluoride vehicles, such as fluoride gels or mouth-rinses, by prolonging the contact of the fluoride with the tooth enamel. [8] A key feature of varnish is the fact that the resin base, in which the fluoride is suspended is tenacious in its adherence to teeth, allowing prolonged fluoride-enamel interaction over time. [9]

The main product deposited on the enamel surface and on subsurface carious lesions after the application of topical vehicles with high fluoride content is calcium fluoride or CaF 2. Topical vehicles with low fluoride concentration tend to deposit fluorapatite or Ca 10 (PO 4) 6 F 2. While fluorapatite remains permanently bound within the crystalline structure of the enamel, calcium fluoride originally was considered to be an undesirable product from topical fluoride treatment because it is readily lost to saliva. Nevertheless, according to studies from the 1980s, it is now known that these compounds may serve as a reservoir of fluoride ions. [8] Under specific thermodynamic circumstances and in the presence of phosphate, part of this CaF 2 can be re-deposited as fluorapatite (i.e., during remineralization). The physical presence of the varnish would facilitate this transformation as fluoride from the varnish may produce a redistribution of ions in the body of a carious lesion, thereby creating a favorable gradient for inward fluoride diffusion and reducing the porosity of the body of the lesion. [8], [10], [11]

Calcium and phosphate technologies currently incorporated into dental products include ACP, CPP-ACP, calcium sodium phosphosilicate, and tricalcium phosphate. The overall intent of these technologies is to increase the amount of available calcium and phosphate, typically together with fluoride. [11]

Hence, with the huge backup of studies on the effective remineralization properties of fluoride varnishes and the introduction of newer fluoride varnishes with added calcium-based remineralizing agents, we took up this study to check the remineralizing efficacy of these modified fluoride varnishes.

In this study, we used two different methods to assess the remineralization potential of the varnishes. The Vickers surface microhardness method helps to qualitatively assess the change in the physical parameter of teeth, i.e., hardness, whereas the SEM-EDAX method helps to quantify the chemical changes that may occur in the teeth following varnish application.

The surface microhardness of all the specimens in Group 1 in the area of the working window was checked with a digital microhardness tester (Model: HMV-2T; Shimadzu Co. Ltd.), with a Vickers elongated diamond pyramid indenter. A load of 50 g was applied to the surface for 5 s. Three such indentations were placed on the surface, and the average value was considered. A built in scaled microscope measured the diagonal length of the indentation, and Vickers values were converted to microhardness values using the following formula:

Vickers hardness (VH) = $1.854 \times F \times 103 / d2$.

where F is the applied test load (N) and d is the average of the indentation diagonals (mm) (De Moor and Veerbeeck, 1998). [12]

The average microhardness value for normal enamel is in the range of 250-360 VHN. [13] In this study, the baseline microhardness values were in the range of 304-355, with the overall average being 347.61 ± 22.88, which was within the standard range. When the baseline microhardness values of the various treatment groups were compared, the difference was found to be statistically nonsignificant which implies that all the pretreatment enamel samples were similar.

In the present study, the specimens were kept in the demineralization solution for 96 h at 37°C, hence creating a subsurface demineralization with an intact surface simulating an early enamel lesion. The concentration of both calcium and phosphate, in the demineralization solution, was at 50% saturation level, causing dissolution of only enamel subsurface which

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simulated the naturally occurring early enamel lesions having intact surface layer. [14] After subjecting the enamel specimens to the demineralization cycle for 96 h, the microhardness values of all the enamel specimens were found to decrease considerably. When the demineralization microhardness values were compared with the baseline values, the difference was found to be statistically significant for all the enamel samples (p < 0.05). This is similar to the results seen in previous studies, in which the surface microhardness of the enamel samples reduces after demineralization. [6],[15],[16],[17],[18],[19] In the comparison of postdemineralization values between the various treatment groups, the difference was found to be statistically nonsignificant which indicates that all the enamel specimens were demineralized to the same extent.

After varnish application and pH cycling, the microhardness values of all the treatment groups except the control group were found to increase considerably indicating mineral gain after varnish application. This again was similar to the results seen in previous studies, wherein the microhardness values increased after varnish application. [6],[15],[16],[17],[18]

Intergroup comparison of the postremineralization microhardness values showed that the values obtained for the varnish groups were statistically significant from those of the control group indicating that varnish application does lead to mineral gain. The comparison between the varnish groups, however, does not reveal a statistically significant difference indicating that all the three varnishes have similar remineralizing properties.

Another method of comparing the remineralizing potentials of the varnishes is by comparing the percentage recovery in microhardness after varnish application. This can be calculated using the following formula: [6]

[INLINE:3]

Using this formula, it was observed that MI Varnish[™] showed the highest microhardness recovery (91.47%), followed by the Flor Opal ® Varnish White group (87.34%) and the Enamel Pro ® Varnish group (86.66%) [Graph 1]. Although the differences in these values were statistically nonsignificant, it goes to show that MI Varnish[™] showed slightly better efficacy as compared to the other two varnishes, but it was not statistically significant. This result is similar to that seen by Lippert et al., 2014, [18] in which Enamel Pro ® Varnish and MI Varnish[™] showed greater microhardness recovery as compared to Flor-Opal ® Varnish White.

[INLINE:1]

Modern prospective caries studies require the measurement of small changes in a tooth's mineral content. One recent technique is SEM-EDAX. EDAX has been used for elemental analysis at the ultrastructural level. It is a microanalytical technique that is used in conjunction with SEM; wherein SEM does the structural analysis, and the elemental analysis is done by EDAX. The principle is based on the energy emitted in the form of X-ray photons when electrons from external sources collide with the atoms in a material, thus generating characteristic X-rays of that element. [20]

The EDAX results are read as peaks for the respective elements on the graph and are quantified as weight % of the total sample. The mean baseline Ca and P values for the sample were $38.43 \pm 2.60\%$ and $21.16 \pm 1.31\%$, respectively, and the mean Ca/P ratio was 1.82 ± 0.50 . The differences in the baseline Ca/P ratios among the various groups were found to be statistically nonsignificant which indicates that the selected samples were uniform in their baseline measurements. These values dropped to $35.24 \pm 2.47\%$, $20.50 \pm 1.25\%$, and 1.72 ± 0.50 after demineralization. For all the four groups, the Ca/P ratio decreased after demineralization, and the difference from the baseline values was statistically significant which is indicative of the mineral loss that occurs after demineralization.

The Ca and P values and the Ca/P ratio increased significantly after varnish application in the three treatment groups to a mean value of $36.49 \pm 2.52\%$, $20.78 \pm 1.23\%$, and 1.79 ± 0.06 . This shows that there is a significant amount of mineral gain on the enamel surfaces after varnish application. This change was statistically significant from that of the control group, thus showing that all the three varnishes are capable of inducing remineralization, which is consistent with the results obtained through microhardness readings. The remineralization potential of the three varnishes was found to be similar as the difference in the postremineralization values of the three varnish groups was statistically nonsignificant.

The EDAX facilitates quantitative assessment of the change in the minerals of the tooth surface and can estimate the mineral changes in a very easy and accurate manner. Although there are very limited studies [21],[22] in which EDAX has been used to study the remineralization potential of fluoride varnishes, the results noted in this study are comparable to the previous studies using regular methods, in which after varnish application, the Ca/P ratio was found to increase which is indicative of the remineralizing capability of the fluoride varnish. Literature review did not reveal a study, in which the remineralizing potential of two or more varnishes was compared using the EDAX method, which makes the present study unique.

SUMMARY AND CONCLUSION

This in vitro study was carried out to compare the remineralizing potential of three different commercially available fluoride varnishes with different formulations. Within the limitations of this in vitro study, the following observations were made:

All the three commercially available varnishes were capable of remineralizing initial enamel lesions that were induced artificiallyAll the three varnishes were able to regain the surface hardness and Ca and P that were lost due to the artificial demineralization processNo difference was noted in the remineralizing efficacy of the varnishes despite their different compositionsMI VarnishTM (CPP-ACP fluoride varnish) showed slightly better recovery in surface microhardness as compared to the other two varnishes. This edge over the other varnishes could be attributed to its formulation. The better efficacy of this varnish needs to be substantiated with further clinical researchThe EDAX method used to quantitatively assess the changes in the mineral content of teeth before and after demineralization and varnish application, proved to be an easy and accurate method of assessment. There are very few studies on varnishes, in which this method has been used and hence the potential of this technique needs to be further explored.Hence, it can be concluded that all the varnishes used in this in vitro study are capable of reversing early enamel lesions and can thus form an integral part of the "Preventive Dentisty" concept.

The main limitation of this study is that being an in vitro study, it cannot exactly replicate the oral environment, in which the action of saliva, the presence of pellicle, masticatory forces, and other such external factors can affect the retention, action, and efficacy of the varnishes. Hence, the remineralization potential of these varnishes needs to be verified through further in vivo studies and randomized controlled trials.

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Conflicts of interest

There are no conflicts of interest.

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