ORIGINAL RESEARCH

Qualitative and Quantitative Profiling of Enamel Remineralizing Potential of Fluoride Varnishes Incorporating Bioactive Glass, Dicalcium Phosphate Dihydrate, and Modified MTA: A Raman Spectroscopic Study

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Abstract

Aim: To evaluate and compare for remineralization potential of enamel at three different time intervals using commercially available MI Varnish and Duraflor Halo varnish [5% sodium fluoride (NaF) varnish] incorporating bioactive glass, dicalcium phosphate dihydrate (DCPD), and biomimetically modified mineral trioxide aggregate (MTA).

Materials and methods: For the study, a buccolingual division into equal halves was done for 64 decoronated premolar crowns. Among these 64 crowns, we mounted 32 in acrylic discs to be used for microhardness and induced white spot lesions (WSLs) measuring 5 × 5 mm on the exposed enamel surface. These samples were divided into four groups of 24 each depending upon the varnish used. Varnish application was done, followed by immersion of these samples for 24 hours in artificial saliva, followed by peeling off of varnish. A pH cycling of 28 days was done after the varnish application before assessing the remineralization of the samples. Evaluation of all these samples was done at three different time intervals, baseline, after demineralization, and post remineralization for microhardness on the enamel surface. Raman spectroscopy was utilized for the measurement of phosphate (P) ion release. Sectioning of these samples to a thickness of 100 µm approximately was done to be viewed under a polarized light microscope.

Results: Bioactive glass incorporated varnish showed the highest microhardness values, mineral content levels, and least depth of lesion posttreatment.

Conclusion: All the experimental varnish showed significant remineralizing potential with the best potency seen with bioactive glass incorporated varnish.

Clinical significance: The nonfluoride agents can be appropriately used in 2 wt% amount to augment the benefits of fluoride.

Keywords: Bioactive glass, Casein phosphopeptide-amorphous calcium phosphate, Demineralization, Dicalcium phosphate dihydrate, Enamel remineralization, Fluoride varnish, MI varnish, Modified mineral trioxide aggregate, Raman spectroscopy.

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Introduction

In the modern era of industrialization, people have neglected health and have revolutionized their lifestyles. Changes in diet and eating behavior mainly portray the changing lifestyle. The major population caught in the spiral of these lifestyle changes are children, who get easy access to readily available processed foods and beverages loaded with hidden sugars. This increased proximity toward sweetened food, in turn, has created a spike in dental caries among children. According to the facts sheet produced by World Health Organization in their 2018 Geneva meeting, 60–90% of children are affected by dental caries globally.[1]

With advancements in the knowledge of the pathology of the caries process, caused by the host, substrate, and tooth surface factors with time resulting in demineralization. It's been easy to control these factors well in hand to reduce caries incidence. Moreover, "caries balance," as stated by Featherstone, is a balance between pathological factors and protective factors governing caries.[2] The change in the delicate balance between these factors leads to caries, which can be effectively applied to revert the early caries process to the so-called "incipient lesions."

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Fluoride (F) is considered to be the main stakeholder for remineralization in dentistry. Various forms are available for the

application of fluorides in dental practice, like toothpaste, gels, rinses, varnish, etc. Among all these available forms, varnish provides the best results. The mechanism behind varnish is that it allows fluoride to stay on the tooth surface for the maximum time and hence acts as a fluoride reservoir and has a slow-release process. But the major drawback of fluoride varnish is its dependence on the biological availability of calcium (Ca) and P for its optimum action. To overcome this hurdle, various modifications have been suggested by adding different nonfluoride remineralizing agents like casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), tricalcium P (TCP), bioactive glass, DCPD, and modified MTA.

Bioactive glass is considered as one of the promising nonfluorides remineralizing agents. During the reaction, a high amount of Ca+ and Phosphate $(PO_4)2-$ ions are released along with a rise in pH, which results in the formation of a Calcium Fluoride (CaF) layer over the tooth surface.[3] Hence, bioactive glass provides a supersaturation of Ca and $(PO_4)2-$ ions on the tooth surface, resulting in optimum remineralization of the tooth. Similarly, a study found 73% of lesions remineralized with bioactive glass, which was higher than another group in the study.[4]

Dicalcium phosphate dihydrate (DCPD) is considered a precursor in enamel and bone formation. This Ca-containing compound helps build a supersaturation Ca level in an aqueous environment.[5] Studies have noticed tremendous Ca incorporation on enamel after DCPD application and elevated Ca levels in plaque for up to 18 hours.[6]

With progress in knowledge and research, biomimetic materials like MTA have been efficiently used. However, a more potent and newer version of MTA is the biomimetically modified MTA added with non-collagenous proteins (NCPs). This is emerging as one of the premium nonfluoride remineralizing agents present in the market.

With this ongoing research and current concepts of remineralization, there is an increased need to launch fluoride vehicles added with nonfluoride agents. Since there is no comprehensive data regarding this aspect of dentistry, through this study, we aim at qualitative and quantitative mineral profiling of fluoride varnish incorporated with bioactive glass, DCPD, and modified MTA.

Materials and Methods

After Institutional Ethical Committee approval, the study was conducted *in vitro*. The teeth samples included in the study were therapeutically extracted premolars. Inclusion criteria were noncarious, nonhypoplastic, no cracks, and nonrestored teeth extracted for orthodontic therapies. While the exclusion criteria were carious and nontherapeutically extracted teeth.

Sample Preparation

The primary steps included cleaning the extracted premolars by removing all the blood and tissues attached with the help of an ultrasonic scaler. The teeth were then placed in 0.1% thymol solution, used as an antifungal agent. This was followed by polishing of the teeth with the help of a pumice slurry and polishing cup, cleaning them with distilled water, and drying the teeth samples with oil-free air by a three-way syringe. Decoronated of teeth at cementoenamel junction was done with a tooth cutting disc mounted on a slow-speed handpiece. The obtained crown was then further divided equally into a mesial and distal half by cutting them buccolingually with the help of a tooth cutting saw.

Varnish Preparation

Experimental varnish was prepared by incorporating 2 wt% of bioactive glass powder, 2 wt% of DCPD, and 2 wt% of modified MTA in Duraflor Halo varnish (5% NaF), respectively. Modified MTA used as mineral trioxide aggregate was incorporated with 3 wt% of polyacrylic acid powder and 8 wt% of sodium tripolyphosphate. The particle size of all the experimental powders was standardized as 10 µ.[7]

These powders were mixed with the varnish at the time of application once the varnish seal was opened.

A total of 96 samples were divided randomly into four groups such that each group comprised 24 samples.

- Group I: MI Varnish (control).
- Group II: About 5% NaF varnish + 2 wt% of bioactive glass powder.
- Group III: About 5% NaF varnish + 2 wt% of DCPD powder.
- Group IV: About 5% NaF varnish + 2 wt% of modified MTA powder.

The 32 teeth samples (to be used for microhardness testing) were mounted in self-polymerizing acrylic of dimensions of 1.2 cm diameter and 0.6 cm height. A plastic slip of 5 × 5 mm was stuck to the middle of the surface enamel of the teeth. Acid resistant nail varnish was applied to the remaining tooth surfaces leaving the area covered by a plastic slip; later, the plastic slip was removed.

Artificial Caries Induction

After the samples were ready, they were subjected to demineralizing solution for the formation of WSL. The demineralizing solution used in the study was inspired by Lara-Carrillo et al.'s work in the paper of 2018. The solution contained 2.2 mM calcium chloride, 2.2 mM monosodium phosphate, 0.05 M acetic acid, and a pH adjusted to 4.6 with 1 M potassium hydroxide. The samples were placed over 96 hours at 37°C to create a white spot lesion of 90 to 120 µm depth.[8] At the end of 96 hours, the teeth samples were washed with distilled water and placed in nonionic artificial saliva.

Varnish Application

Three experimental varnishes and one control varnish were used in this study. The varnish used for the control group was commercially available MI varnish. The three experimental varnishes included 5% NaF varnish (Duraflor Halo Varnish, Medicom, United States of America) incorporated with 2 wt% of bioactive glass powder, DCPD, and modified MTA. All four varnishes were applied to the respective samples using an applicator brush according to the manufacturer's instructions. The type of application method used in this study was an intensive application method (three times a week).[9] Every time the varnish was applied, it was kept in artificial saliva for 24 hours, followed by peeling off the varnish.

Further, the 32 samples were tested for microhardness at the following three different time intervals:

- Before the creation of artificial lesion (baseline).
- After the creation of artificial lesion.
- After remineralization of the artificial lesion.

Vickers microhardness testing machine was used to test these samples with a load of 300 gm and a dwell time of 15 seconds. The 32 samples of Raman spectroscopic analysis were also polished with silicon carbide paper of different grit prior to the application of nail varnish and induction of artificial caries to achieve a flat surface. Jobin-Yvon Ramantor HG25 was used to

record Raman spectra equipped with a double monochromator and a photon counting module. The excitation source of this machine was an argon ion laser. The wavelength of the laser to which the machine tuned was 514.5 nm. The outpower was fixed to 100 W to avoid surface heating. A light optical microscope of Micro-Raman spectrometer was used to focus the laser beam on the object, and the focal size-adjusted was 10 µm. The analysis gave peaks of intensity at different Raman shifts. These peaks of P ion were compared for intensity. To understand the subsurface changes, a polarized light microscope was used. As discussed earlier, the single tooth sample for the depth profile was divided buccolingually equally into mesial and distal half. One half of the same tooth was used to determine the depth profile after induction of the artificial lesion, and the second half of the same tooth was used after the remineralization of artificial caries. These tooth samples were cut mesiodistally from the center of the lesion to prepare a sample of thickness 100 µ with Silverstone-Taylor microtome. These samples were mounted on a microscopic glass slide. These sections were evaluated and photographed under a polarized light microscope, Olympus TL4. An imaging system was used to quantify demineralized areas. Quantitative analysis of measurement of the depth of the lesion was done using ImageJ software. Three subsequent readings were taken for each sample, and a mean of these three readings was used as the final reading of each sample.

Results

Statistical analysis was performed using Statistical Package for the Social Science (SPSS) version 21 for Windows (SPSS Inc., Chicago, Illinois, United States of America). Descriptive quantitative data was expressed in mean and standard deviation, respectively. Data normality was checked by using Shapiro–Wilk test. The confidence interval was set at 95%, and the probability of α error (level of significance) was set at 5%. The power of the study was set at 80%. Overall intergroup comparisons of microhardness, mineral profile, and depth profile among four groups at each study interval were made using one-way analysis of variance (ANOVA) *F*-test, followed by Tukey's *post hoc* test for multiple pairwise comparisons. Intragroup comparison in relation to each parameter (microhardness, mineral profile, and depth profile) was made at different time intervals using one-way ANOVA *F*-test, followed by Tukey's *post hoc* test for multiple pairwise comparisons. Intergroup comparison between the experimental and control group was made using an unpaired *t*-test.

Intragroup Comparison for Microhardness at Three Different Time Intervals

The intragroup comparison showed the maximum microhardness recovery by the bioactive glass group (mean 233.22), indicating the best remineralization potential, followed by a lesser microhardness but significant remineralizing efficacy by MI Varnish, DCPD, and modified MTA as shown in Table 1 and Figure 1.

Intragroup Comparison for Mineral Profile at Three Different Time Intervals

The intragroup comparison showed the maximum recovery of P ion by the bioactive glass group (mean 12206), indicating the best remineralization potential, followed by a lesser P ion level but significant remineralizing efficacy by MI Varnish, DCPD, and modified MTA as shown in Table 2 and Figure 2.

Intragroup Comparison for Depth Profile at Three Different Time Intervals

The intragroup comparison showed the maximum decrease in lesion depth by the bioactive glass group (mean 292.55), indicating the best remineralization potential, followed by a higher lesion depth but significant remineralizing efficacy by MI Varnish, DCPD, and modified MTA as shown in Table 3 and Figure 3.

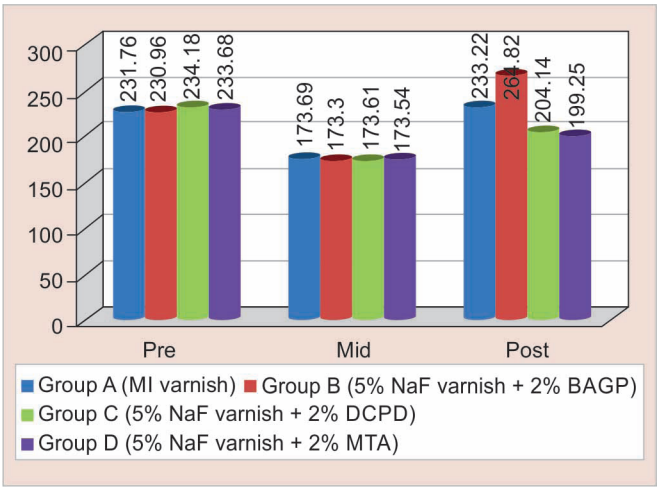


Fig. 1: Comparison of microhardness among the groups at three different time intervals

Table 1: Comparison of microhardness among the groups among three different time intervals

	Premean [standard deviation (SD)]	*Midmean (SD)*	*Postmean (SD)*
Group I (MI Varnish)	231.76 (5.98)	173.69 (5.33)	233.22 (3.68)
Group II (5% NaF varnish + 2% BAGP)	230.96 (4.19)	173.38 (5.13)	264.82 (3.62)
Group III (5% NaF varnish + 2% DCPD)	234.18 (4.02)	173.61 (3.48)	204.14 (3.35)
Group IV (5% NaF varnish + 2% MTA)	233.68 (3.72)	173.54 (5.49)	199.25 (5.63)
One-way Anova *F*-test value	$F = 0.896$	$F = 0.006$	$F = 420.707$
p-value (overall)	$p = 0.455$ (NS)	$p = 0.999$	$p < 0.001^{b}$

$p > 0.005$, no significant difference; [a], $p < 0.05$–significant; [b], $p < 0.001$—highly significant; ^, *p*-value calculated using one-way ANOVA *F*-test; BAGP, Bioactive Glass Powder; NS, Non-standard

Table 2: Comparison of mineral profile using Raman spectroscopy among the groups at three different time intervals

	Premean (SD)	*Mid mean (SD)*	*Postmean (SD)*
Group I (MI Varnish)	12268 (5.96)	8352 (4.56)	12206 (3.43)
Group II (5% NaF varnish + 2% BAGP)	12265 (2.56)	8306 (6.41)	12235 (3.24)
Group III (5% NaF varnish + 2% DCPD)	12267 (4.01)	8338 (3.78)	12170 (4.69)
Group IV (5% NaF varnish + 2% MTA)	12270 (4.98)	8339 (3.62)	12129 (3.81)
One-way ANOVA *F*-test value	$F = 1.475$	$F = 1.402$	$F = 1152.0$
p-value (overall)	$p = 0.243$ (NS)	$p = 0.263$ (NS)	$p < 0.001$[b]

$p > 0.005$, no significant difference; [a], $p < 0.05$—significant; [b], $p < 0.001$—highly significant; ^, *p*-value calculated using one-way ANOVA *F*-test; NS, Non-standard

Table 3: Comparison of depth profile measurements among the groups at the demineralization and remineralization phase

	Demineralization mean (SD)	*Remineralization mean (SD)*
Group I (MI Varnish)	341.14 (3.26)	292.55 (3.32)
Group II (5% NaF varnish + 2% BAGP)	341.19 (2.97)	251.62 (3.23)
Group III (5% NaF varnish + 2% DCPD)	340.35 (4.03)	323.86 (3.19)
Group IV (5% NaF varnish + 2% MTA)	340.30 (3.35)	333.01 (2.24)
One-way ANOVA *F*-test value	$F = 0.160$	$F = 1176.0$
p-value (overall)	$p = 0.923$ (NS)	$p < 0.001$[b]

$p > 0.005$, no significant difference; [a], $p < 0.05$—significant; [b], $p < 0.001$—highly significant; ^, *p*-value calculated using one-way ANOVA *F*-test; BAGP, Bioactive Glass Powder

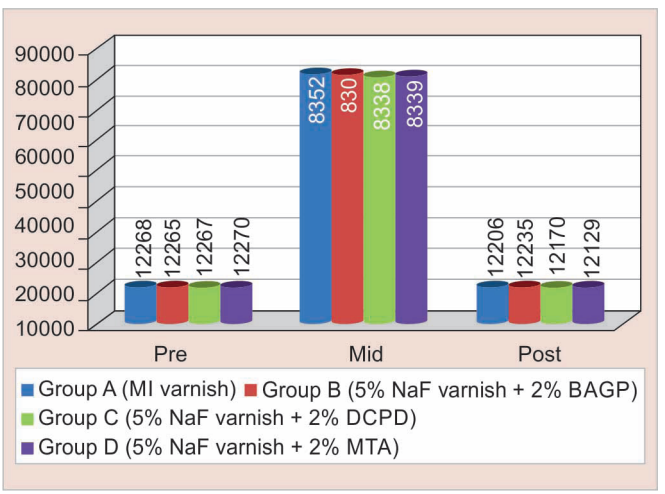


Fig. 2: Comparison of mineral profile using Raman spectroscopy among the groups at three different time intervals

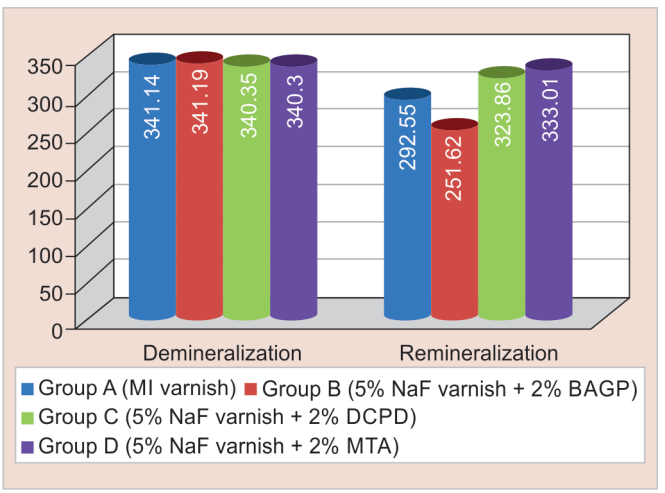


Fig. 3: Comparison of mineral profile using polarized light microscopy among the groups at three different time intervals

Intergroup Pairwise Comparison for Microhardness

Intergroup pairwise comparison showed a highly significant difference for microhardness values after treatment with varnish for bioactive glass compared to MI Varnish, bioactive glass compared to DCPD, and modified MTA, as shown in Table 4 and Figure 4. Although no significant difference was noted between DCPD and the modified MTA group.

Intergroup Pairwise Comparison for Mineral Profile

Figure 5 depicts the Raman spectra for all four groups.

Intergroup pairwise comparison showed a highly significant difference for P ion levels values after treatment with varnish for bioactive glass compared to MI varnish, bioactive glass compared to DCPD and modified MTA, as shown in Table 5 and Figure 6.

Table 4: Pairwise comparison of microhardness between the groups

Pair	*Difference*	*p-value*
I vs II	31.6	$p < 0.001^b$
I vs III	29.08	$p < 0.001^b$
I vs IV	33.97	$p < 0.001^b$
II vs III	60.68	$p < 0.001^b$
II vs IV	65.57	$p < 0.001^b$
III vs IV	4.88	$p = 0.113$ (NS)

$p > 0.005$, no significant difference; [a], $p < 0.05$—significant; [b], $p < 0.001$—highly significant; ^, *p*-value calculated using Tukey's *post hoc* test for intergroup pairwise comparisons

Table 5: Pairwise comparison of mineral profile using Raman spectroscopy among the groups

Groups	*Pair*	*Difference*	*p-value*
Post	I vs II	29.71	$p < 0.001^b$
	I vs III	35.81	$p < 0.001^b$
	I vs IV	76.97	$p < 0.001^b$
	II vs III	65.53	$p < 0.001^b$
	II vs IV	106.69	$p < 0.001^b$
	III vs IV	41.16	$p < 0.001^b$

$p > 0.00$, no significant difference; [a], $p < 0.05$—significant; [b], $p < 0.001$—highly significant; ^, *p*-value calculated using Tukey's *post hoc* test for intergroup pairwise comparisons

Moreover, a highly significant difference was also noted between DCPD and modified MTA group for Raman spectroscopy.

Intergroup Pairwise Comparison for Depth Profile

Intergroup pairwise comparison showed a highly significant difference for a decrease in lesion depth values after treatment with varnish for bioactive glass compared to MI Varnish, bioactive glass compared to DCPD, and modified MTA, as well as shown in Table 6 and Figure 7. But no significant difference was noted between DCPD and the modified MTA group. Lesion depth could be observed groupwise from Figure 8.

Discussion

This study compared the experimental varnishes by adding 2 wt% of bioactive glass, DCPD, and modified MTA powder to 5% NaF varnish, as the commercially available formulation of varnish added with a nonfluoride remineralizing agent, MI Varnish has this composition of 5% NaF + 2% CPP-ACP, in the present study, we chose MI Varnish as the control group among other commercially available similar formulations like Clinpro White varnish containing TCP, as Tuloglu et al. concluded in their study that MI Varnish showed best caries resistance among Duraphat and Clinpro White Varnish.[10]

This experiment used demineralizing and remineralizing solutions advocated by Lara-Carrillo et al. The samples were demineralized for 96 hours to achieve a lesion depth of at least 120 µm.[7] The samples were subjected to pH cycling for 28 days, as advocated by Amaechi, the pH cycling protocol to best achieve remineralization after treatment with varnish.[11] Perhaps, following the best pH cycling protocol to mimic the oral environment could never mimic completely because of various factors like speed, saliva flow rate, the buffering capacity of saliva, eating habits of individuals, etc.

Microhardness testing was done with the help of Vickers microhardness testing machine for all samples at three different

Table 6: Pairwise comparison of depth profile measurements among the groups

Groups	*Pair*	*Difference*	*p-value*
Remineralization	I vs II	40.92	$p < 0.001^b$
	I vs III	31.31	$p < 0.001^b$
	I vs IV	40.46	$p < 0.001^b$
	II vs III	72.23	$p < 0.001^b$
	II vs IV	81.38	$p < 0.001^b$
	III vs IV	9.15	$p = 0.063$

$p > 0.005$, no significant difference; [a], $p < 0.05$—significant; [b], $p < 0.001$—highly significant; ^, *p*-value calculated using Tukey's *post hoc* test for intergroup pairwise comparisons

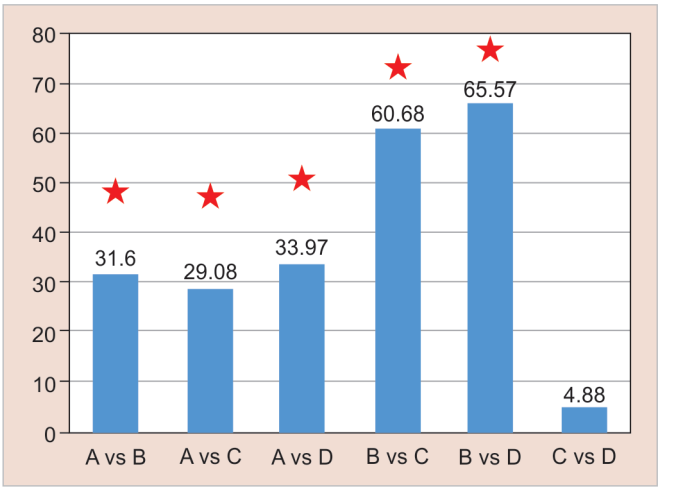


Fig. 4: Pairwise comparison of microhardness between the groups

time intervals. From the results, it could be observed that baseline microhardness values are almost in accordance with all groups, with very similar and identical decreases in microhardness after demineralization. Still, the microhardness of remineralized samples showed maximum improvement for bioactive glass incorporated varnish. Our results are in accordance with Mehta et al., who concluded a better increase in microhardness of samples after treatment with SHY-NM (bioactive glass containing toothpaste).[12] The observation could be due to the formation of crystallite hydroxycarbonate apatite after the reaction of bioactive glass, which is more stable than ACP and acts as a reservoir to release more Ca and P ions. All other groups except DCPD and modified MTA showed a significant difference in microhardness values after remineralization, but DCPD and modified MTA were able to remineralize artificial caries.

Raman spectroscopic analysis was done for mineral profiling of all samples. From the results, the peaks were observed in a band of 540-1040-1 cm, among which the P peak corresponds to the 960-1 cm Raman shift. The graphs could depict the changes in the intensity of peaks at baseline, after artificial caries induction and post remineralization. This signifies the remineralization potential of all experimental groups. Among all the groups, the highly significant post remineralization values were shown by bioactive glass incorporated varnish. The present study results are in accordance with Hassanein and El-Brolossy and Gjorgievska and Nicholson, which showed much better Ca and P release for remineralization from bioactive glass.[13,14] The potential consideration could be the protective layer usually formed in

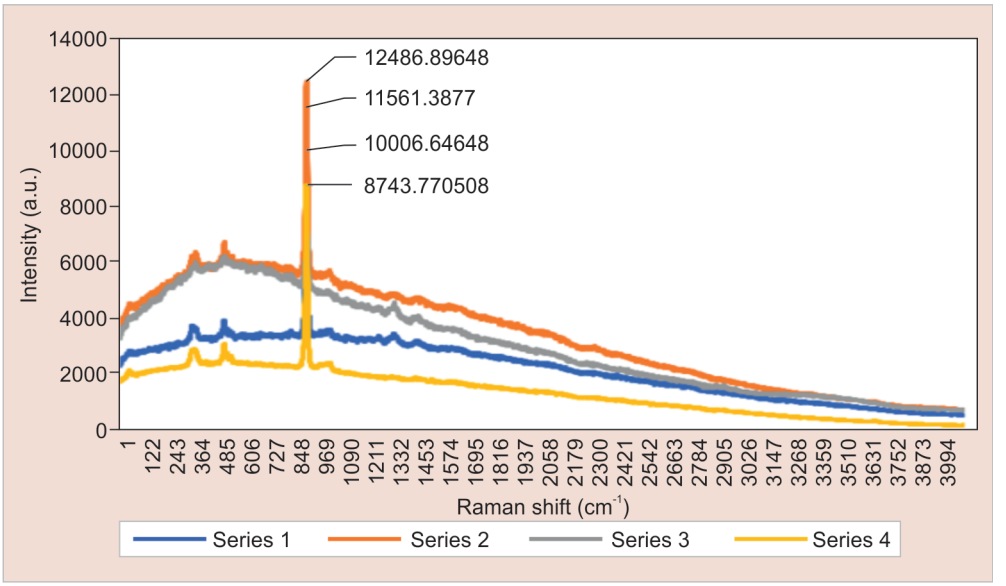


Fig. 5: Comparative change in P levels in Raman spectroscopy after remineralization with experimental varnish

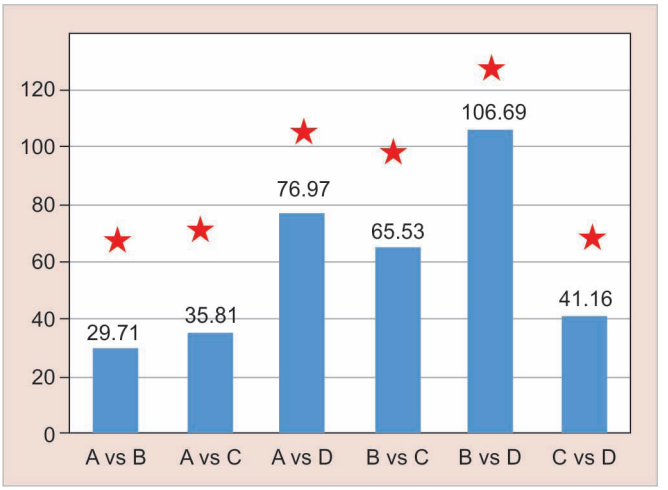


Fig. 6: Pairwise comparison of mineral profile using Raman Spectroscopy among the groups

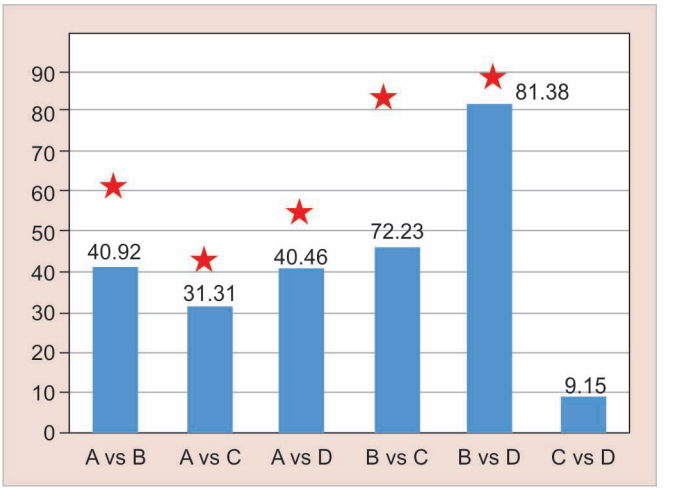
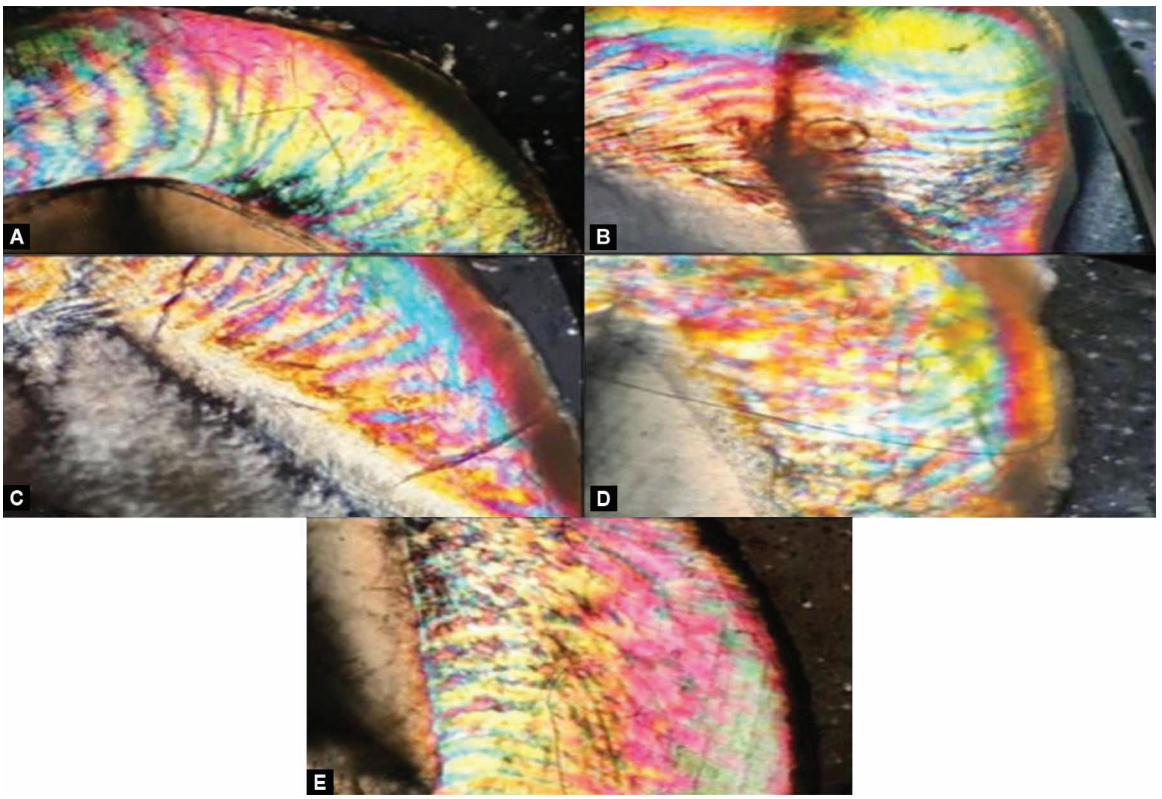


Fig. 7: Pairwise comparison of depth profile measurements among the groups

the MI Varnish as it forms ACP, which may take a longer time to dissolve the layer and increase the Ca and P levels. Besides, as mixed with saliva, bioactive glass reacts with hydrogen ions and hence drops the pH, which possibly helps in better precipitation of ions. DCPD also showed significant remineralization by depicting increased values of P ions. This result stays in accordance with Sullivan et al., who demonstrated a substantial release of Ca in their study by labeling the Ca.[6] The reason behind the decreased potential of DCPD than bioactive glass could be the only release of more Ca and not P, which somewhere acts as a stumbling block to its remineralization potential. Further, the modified MTA also remineralized the samples significantly but less than all other groups. The result of its remineralization potential is in accordance with Qi et al. which showed better remineralization of bovine enamel slabs with biomimetically modified MTA.[7] The grounds for the downscale result for modified MTA could be the less availability of NCPs as we had used the composition of modified MTA as described by Qi et al., but this may require a higher weight percent of NCPs for its better remineralizing action.[8] Moreover, we had used only 2 wt% of modified MTA in fluoride varnish, which may not be sufficient in amount for its optimum action.

Polarized light microscopy was used to evaluate the subsurface changes after artificial caries induction and remineralization in all samples. All the experimental varnishes showed a significant reduction in lesion depth for all samples except for the modified MTA group. The bioactive glass group saw the maximum reduction in the depth of the lesion. Similar results were seen with Rajan et al. and Prabhakar et al. as they concluded better mineral deposition at the subsurface level by the bioactive glass.[15,16] The viable origin for the observation may be the ability of bioactive glass to deposit at the mineral layer on the dentin, which eventually occludes dentinal tubules by 80%, and hence is also used to reduce hypersensitivity. However, MI Varnish containing CPP-ACP could not depict this property clearly and thus acts as a slow delivery system of Ca, P, and F only for enamel.



Figs 8A to E: Polarized Light Microscopy images of the enamel surface for measurement of depth of remineralization. (A) Demineralized sample; (B) MI Varnish (control); (C) Duraflor + bioactive glass; (D) Duraflor + DCPD; (E) Duraflor + modified MTA

According to the present study, the assessment of microhardness at different time intervals portrayed the highest topographical values for bioactive glass varnish. The MI Varnish group depicted the second highest microhardness values. While the DCPD and modified MTA could remineralize but not significantly among themselves. Raman spectroscopic analysis showed the highest mineral recovery in the bioactive glass group in the present study. The second highest group for P levels was in MI Varnish. Moreover, DCPD and modified MTA also significantly revived the mineral losses compared to MI Varnish and bioactive glass and among themselves. In the present study, polarized light microscopic evaluation depicted the highest decrease in lesion depth and reversal of caries in the bioactive glass. The highest group to reduce lesion depth was MI Varnish.

In the present study, we compared and evaluated the remineralizing potential of fluoride varnish incorporating experimental powders, bioactive glass, dicalcium phosphate, and modified MTA. This is one of the pioneer studies which incorporated DCPD and modified MTA in fluoride varnish, considering calcium and P supplements for remineralization, and gives topographical enamel evaluation both quantitatively and qualitatively added to the subsurface evaluation. Thus, it contributes significantly to the field of preventive dentistry with the help of appropriate evaluation parameters. These tested experimental varnishes can be used to treat early WSLs effectively. The varnishes have shown better efficacy for remineralization than commercially available options. Hence, it can be used as an elite substitute in pediatric dentistry, which guarantees substantial remineralization and a longer duration of recall would visit, paving the way to bright smiles. Being the instigate study to incorporate various nonfluoride supplements, further *in vivo* studies and clinical trials are required.

Conclusion

The nonfluoride agents can be appropriately used in 2 wt% amounts to augment the benefits of preventive treatment without hampering the fundamental mechanism of remineralization of fluoride varnishes. Bioactive glass varnish has the best potential to restore microhardness and recover lost minerals. The paucity of literature in exploring the full scope of this emerging tool paves the way for clinically oriented research to harness its complete potential and enhance the remineralization strategies available in the market today.

Clinical Significance

To the best of our knowledge, the present study is one of the founding studies to have compared four varnishes with novel compositions for their inclusive effect on remineralization and fluoride release. When compared to commercially available varnishes, positive results of the therapy by experimental groups could be an effective option in enhancing the process of remineralization of WSLs *in vivo*. Although light amplification by stimulated emission of radiation therapy has been researched upon, nonthermal plasma is an upcoming trend in dentistry, still simple techniques have their own sweet place.

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