**INTRODUCTION**

Dental caries is among the key cause of tooth loss in adults as well as in children. There have been numerous studies conducted in the past which have helped us to reduce the prevalence of dental caries in developed countries, but it is still a major concern for tooth loss in developing countries.\(^1\)

According to Featherstone (1999),\(^2\) dental caries is a process of alternating loss and gain of tooth minerals. Contrary to the past studies, dental caries is now considered a reversible process that can be arrested or reversed (Murdoch-Kinch and McLean, 2003; Featherstone, 2006).\(^3\) There have been various studies conducted which indicated that “white spot” lesions can be arrested or in few cases remineralized with the help of a surplus amount of fluoride, calcium, and phosphate ions in saliva. In the past, the surgical approach for early surface lesions made the tooth crippled and posed irreparable damage. However, biological approaches are now focusing on the application of remineralizing agents to early carious lesions to promote remineralization.\(^1\)

“Remineralization” has been described as a process of increase in the mineral amount in saliva which precipitates over enamel surfaces. Therefore, if we provide these remineralizing ions from an external source, it facilitates the deposition of these ions into crystal voids which are accessible spaces in a crystal formed by loss of enamel surface which leads to an increase in the net mineral amount over enamel surface.

Various remineralizing agents are available in the form of dentifrices. This study compares the remineralization potential of CPP-ACFP, novamin, and nanohydroxyapatite to professionally applied fluoride varnish in both primary and deciduous teeth.

**MATERIALS AND METHODS**

Eighty extracted premolars and 80 extracted or exfoliated primary incisors were included in the study (Figs 1 to 5).

**Inclusion Criteria**

- Teeth with intact enamel surfaces, no decalcification.
- Teeth are not affected by fluorosis.
- Teeth extracted due to reasons other than dental caries.
- Naturally exfoliated teeth.
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**Exclusion Criteria**
- Teeth with caries or restorations.
- Teeth with developmental defects.
- Discolored teeth.
- Fractured teeth.
- Teeth having cracked areas.

**Preparation of Window**
All samples of permanent and primary teeth were covered with nail varnish leaving two windows of $3 \times 3$ mm and $2 \times 2$ mm, respectively, on the buccal surface which was covered by a square piece of plaster adhesive tape.

**Preparation of Samples for Demineralization and Remineralization**
The masking tape was removed once the varnish dried. The teeth were suspended in a demineralizing solution which was a mixture of calcium chloride (2.0 mmol/L), trisodium phosphate (2.0 mmol/L) in acetate buffer (75 mmol/L) solution at pH 4.6 for 4 days, which created artificial caries like lesions on the exposed surfaces.

The teeth were then removed from the demineralizing solution and washed. Both the deciduous and permanent teeth were then cleaved into four groups equally.

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**Fig. 1:** Materials used

**Fig. 2:** Experimental materials

**Figs 3A and B:** Teeth specimens

**Fig. 4:** Windows created
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Fig. 5A and B: Application of nail varnish

Fig. 6: Prepared demineralising solution and artificial saliva

Group I—Fluoridated varnish group (Fluorprotector; Ivoclar Vivadent).
Group II—(CPP-ACPF) G C Tooth mousse plus group (Recaldent; GC Corp; Japan).
Group III—Dentifrice containing nanohydroxyapatite group (ACLAIM).
Group IV—Bioactive glass group (sodium-calcium phosphosilicate) (NOVAMIN).

Among the two windows created on each tooth, the upper one was used for the baseline value of demineralization while the lower window was used for remineralization in each group. The experimental materials were applied to the lower window of the teeth for the next 40 days. Fluoride varnish was applied once daily while the other materials were applied twice daily for 4 minutes.

The samples were sectioned longitudinally after mounting them in self-cure acrylic resin so that each section included the demineralized window surface as well as the remineralized area with a microtome. Each specimen that was produced was 150 μm thick. The sections were finished and then painted with 0.1 mM Rhodamine B solution for 1 hour. They were then washed thoroughly with phosphate buffer solution. After treatment, the samples were mounted on frosted glass slides with 80% glycerol mountant for further analysis through the confocal laser scanning microscope.

The images were captured from the buccal surface that is one each from either side of the midpoint measured from the occluso-cervical length of the tooth at (5x) magnification and Argon laser was used at 488 nm wavelength for excitation and emission range of 498–514 nm. Using the Leica TCS SL in-built software, the captured images were calibrated for the linear depth of fluorescence and the average/total fluorescence of the lesion. These values were noted, tabulated, and were compared with the evaluated demineralized values of the same specimen and were subjected to statistical analysis (Figs 6 to 11).

Statistical Analysis
The recorded values are presented as mean ± standard deviation, range, and percentage changes. The recorded values are statistically analyzed using ANOVA, Fisher’s T-test, and Bonferroni test.

For all the tests, a p value of 0.001 or less was accepted for statistical significance.

Results
Table 1 shows the mean values of depth of demineralization, depth of remineralization, and the depth of demineralized area after remineralization of all the groups for both permanent and deciduous teeth, respectively. It also compares the amount of remineralization that has occurred in each group between permanent and deciduous teeth and found that the amount of remineralization in deciduous teeth was significantly more than that of permanent teeth for each group.

When fluoride varnish was compared with casein phosphopeptide amorphous calcium phosphate fluoride (CPP-ACPF) paste, novamin paste, and paste containing nanohydroxyapatite in permanent teeth, it showed a statistically significant increased remineralization potential as compared to CPP-ACPF and nanohydroxyapatite, but the difference in remineralization potential between fluoride varnish and novamin paste was not statistically significant (Table 2).

While in deciduous teeth group I sample showed the highest remineralization potential followed by novamin, CPP-ACPF, and nanohydroxyapatite with a significant difference.

Discussion
Fluoride has been the mainstay in the management of caries lesions for white spot lesions. Fluoride varnish is used to extend the period of contact of fluoride ions with the enamel surface, enhancing the formation of fluorapatite and reducing the solubility of enamel in acid. Arends and Schuthof showed that silane fluoride of Fluorprotector reacts with water to produce a considerable amount of hydrofluoric acid (HF) which penetrates enamel more readily than fluoride ions and shows better remineralization potential. Various studies in the past have also shown that varnishes provide fluoride more efficiently than other topical agents, with reductions in caries...
ranging from 50 to 70%. Hence, in this study, fluoride varnish was selected as the professionally applied remineralizing agent.

In 1981, it was found that milk, milk concentrates, powders, and cheese help prevent dental caries in animals and in situ caries models. It was found that bovine CPP stabilizes calcium and phosphate ions in metastable solution in both acidic and basic pH solutions and even in the presence of fluoride ions forming fluorapatite or fluorhydroxyapatite crystals on enamel lesions. Hence, in this study, CPP-ACFP was preferred over CPP-ACP to check its remineralizing efficacy and compare it with other remineralizing agents.

Bioactive glass is made of amorphous sodium-calcium phosphosilicate. It is a ceramic material that exchanges sodium ions with hydrogen cations (in the form of H$_3$O$^+$) in the presence of saliva and this brings about the release of calcium and phosphate (PO$_4^{3-}$) ions from the glass. These calcium and phosphate ions form a calcium phosphate layer hydroxycarbonate apatite (HCA) directly, without the intermediate ACP phase. These particles also attach to the tooth surface and continue to release ions and remineralize the tooth surface after the initial application.

Nanohydroxyapatite crystals as studied in the past have shown the potential to remineralize the decayed tooth surfaces. It also has
bioactive and biocompatible properties. It was shown that when nano-HA crystals penetrate the enamel pores, they act as a template that continuously attracts a large amount of Ca$^{2+}$ and PO$_4^{3-}$ ions from the remineralization solution. This in turn will promote crystal integrity and growth.8

In the present study, the highest amount of remineralization was shown by fluoride varnish followed by sodium-calcium phosphosilicate, CPP-ACPF paste, and nanohydroxyapatite in both permanent and deciduous teeth.

In this study, there was no significant difference between the remineralization potential of professionally applied fluoride varnish and novamin in permanent teeth.

Mehta et al. found that the application of novamin more effectively remineralized the carious lesion when compared with dentifrices containing CPP-ACP. She found that the deposits formed by novamin appeared to be more compact and intimately attached to the enamel surface which was larger and more angular. Whereas the deposits formed by CPP-ACP were smaller and amorphous. So, we can also suggest that there is a high amount of hardness for novamin as compared to CPP-ACFP in the above-conducted study.

In this study, nanohydroxyapatite paste was used for 40 days and due to the low solubility of pure hydroxyapatite, not enough Ca$^{2+}$ and PO$_4^{3-}$ were available to increase the stability of hydroxyapatite in the enamel and promote remineralization.9 This might be the reason for the low remineralization efficacy of nanohydroxyapatite when compared to novamin, CPP-ACFP, and fluoride varnish in both deciduous and permanent teeth.

It was also observed in the above study that the amount of remineralization for all the groups was found to be higher in deciduous teeth when compared to permanent teeth. This may be due to differences in the structures that exist between permanent and deciduous teeth enamel.

The enamel of deciduous teeth is thinner, more porous, less mineralized, and has more carbonate, less phosphorus, and calcium phosphates in its composition. It is plausible to state that the overlying enamel in dentine caries lesion breaks more easily in primary than in permanent teeth. Therefore, fewer non-evident caries lesions would occur in primary teeth than in permanent ones. As the degree of porosity is higher in deciduous enamel, it leads to an increase in permeability which may be the reason for a high amount of exchange of ions which would lead to a high amount of demineralization and remineralization in deciduous teeth when compared to permanent teeth.10–12

### Table 1: Comparison of mean values of permanent and deciduous teeth for each group by Fisher pair t-test

| Study groups | Permanent | Deciduous |
|--------------|-----------|-----------|
|              | Depth of demineralized area (μ) | Depth of remineralized area (μ) | Depth of demineralized area after remineralization (μ) | Test value | p value |
| Group I      | 146.63    | 112.90    | 33.73      | 157.67 | 126.88 | 30.79 | 6.423 | p < 0.01 |
| Group II     | 148.16    | 109.85    | 38.31      | 158.85 | 122.55 | 36.30 | 2.122 | p < 0.01 |
| Group III    | 148.38    | 106.16    | 42.22      | 160.28 | 118.36 | 41.92 | 3.657 | p < 0.01 |
| Group IV     | 148.61    | 113.64    | 34.97      | 159.46 | 125.98 | 33.48 | 7.556 | p < 0.01 |

### Table 2: Comparison of the mean depth of remineralization between groups using Bonferroni’s test in permanent and deciduous teeth

| (i) group | (J) group | Test value | p value |
|-----------|-----------|------------|---------|
| Group I   | Group II  | 5.319      | < 0.001 |
| Group III | Group I   | 9.486      | < 0.001 |
| Group IV  | Group I   | 1.627      | 0.115   |
| Group II  | Group I   | −5.319     | < 0.001 |
| Group III | Group I   | 4.238      | < 0.001 |
| Group IV  | Group I   | 4.119      | < 0.001 |
| Group III | Group II  | −9.486     | < 0.001 |
| Group III | Group IV  | −4.238     | < 0.001 |
|          | Group III | 8.615      | < 0.001 |

| Deciduous teeth | (i) group | (J) group | Test value | p value |
|-----------------|-----------|-----------|------------|---------|
| Group I         | Group II  | 7.857     | < 0.001   |
| Group III       | Group I   | 14.055    | < 0.001   |
| Group IV        | Group I   | 3.614     | < 0.001   |
| Group II        | Group I   | −7.857    | < 0.001   |
| Group III       | Group I   | 9.062     | < 0.001   |
| Group IV        | Group I   | 4.873     | < 0.001   |
| Group III       | Group II  | −14.055   | < 0.001   |
| Group III       | Group II  | −9.062    | < 0.001   |
| Group IV        | Group II  | 12.364    | < 0.001   |

Fig. 11: Image after remineralization under confocal microscopy
Therefore, all the four materials used in the study showed remineralization potential which was significantly higher in the deciduous teeth when compared to permanent teeth for all the materials tested.

**CONCLUSION**

With certain limitations of this study, it can be suggested that fluoride varnish is the most effective among the four agents tested for the remineralization of an artificial carious lesion in both permanent and deciduous teeth. However, it is professionally applied, hence bioactive glass, a self-applied remineralizing agent can also be used as an alternative as there was no statistically significant difference between fluoride varnish and bioactive glass.

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