
Dr. Shabista Jabi1, Dr. Swati Dwivedi2, Dr. Monika Koul3, Dr. Ahsan Abdullah4, Dr. Vinod Upadhyay5, Dr Swati Goley6

1Senior lecturer, Department Paediatric & Preventive Dentistry, Narayan swami dental college and hospital.
2Professor, Pediatric & Preventive Dentistry, Career Postgraduate Institute of Dental Sciences & Hospital, Lucknow
3Professor, (HOD), Pediatric & Preventive Dentistry, Career Postgraduate Institute of Dental Sciences & Hospital, Lucknow
4Reader, Paediatric & Preventive Dentistry, Career Postgraduate Institute of Dental Sciences & Hospital, Lucknow.
5Professor, Career Postgraduate Institute of Dental Sciences & Hospital, Lucknow.
6Senior lecturer, Department of oral medicine and radiology, Narayan Swami Dental College And Hospital, Deharadoon

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Background- Early treatment of white spot lesions is essential to prevent the progression of the lesion.
Aim - To evaluate and compare the remineralizing potential of CPP-ACP with fluoride, Icon and S-PRG fillers using atomic absorption spectroscopy and calorimetric method.
Materials and methods- Sixty sound human premolars were divided into three groups (A, B & C), consisting of 20 samples each White spot lesions (WSLs) were established on the window (4x4 mm²) created on the buccal surfaces of the samples. Samples in group A was treated with CPP-ACP with fluoride, those of group B was treated with Icon, while group C was treated with S-PRG Fillers. The sample teeth were immersed in a demineralizing solution for 4 days. The samples were subjected for loss of mineral content (wt %) i.e of calcium using atomic absorption spectroscopy and phosphorus using calorimetric method.
Results- Statistical analysis was conducted using one-way analysis of variance, Tukey’s and paired t-tests (P < 0.05). Result of the study showed that there was a statistically significant difference between the three groups, where Icon (group B) had the highest remineralizing potential followed by CPP-ACP with fluoride (Group A) then S-PRG Fillers (Group C).
Conclusion- There was a significant difference in remineralizing potential of Icon, CPP-ACP with fluoride and S-PRG Fillers. Icon appears to be an effective technique in the remineralization of white spot lesion.

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Corresponding Author: Dr. Shabista Jabi, Senior lecturer, Department Paediatric & Preventive Dentistry, Narayan swami dental college and hospital.
INTRODUCTION:
Dental caries is one of the oldest diseases since mankind and it is often described as “pandemic” disease due to its high prevalence. Signs of the caries process are the first molecular change in the apatite crystals of the tooth, to a visible white spot lesion (WSL), or even eventual cavitation. It occurs as a result of cyclic demineralization and remineralization of enamel due to altered pH levels. Demineralization is defined as the process by which minerals (calcium and phosphate ions) are removed from the tooth. Demineralization occurs at low pH when the oral environment is under saturated with mineral ions, comparative to a tooth’s mineral content. Whereas, remineralization of tooth enamel is defined as the process whereby calcium and phosphate ions are supplied from an external source to promote ion-deposition on demineralized enamel crystals to produce mineral gain.

White spot lesions occur when the pathogenic bacteria have breached the enamel layer and organic acids produced by the bacteria have leached out a certain amount of calcium and phosphate ions that may or may not be replaced naturally by the remineralization process. These White Spot Lesions (WSL) are usually formed in patients who have undergone fixed orthodontic treatment. Other factors include xerostomia, high caries index, fluorosis and developmental hypoplasia. The white spot is most easily observed when the enamel is thoroughly dried. The treatment of such lesions should aim upon both improving the aesthetics and prevention of caries progression.

The first line of management of white spot is remineralization. The development of white spot lesions can be slowed or even arrested by various procedures such as removal of etiologic factors like maintaining oral hygiene and use of remineralizing agents such as topical fluorides, CPP-ACP, Bioactive glass, ACP technology, Tri-calcium phosphate, Xylitol, Icon, SDF and S-PRG fillers. Calcium phosphate remineralization technology based on CPP-ACP with fluoride (Mi Varnish) has been recently developed, where CPP stabilizes high concentrations of calcium and phosphate ions, together with fluoride ions, at the tooth surface by adhering to pellicle and plaque, thus preventing demineralization and enhancing remineralization. Icon is a new minimally invasive technique for treating white spot lesions by caries infiltration. Icon prevents further progression of initial enamel caries lesions and occludes the microporosities within the lesion by infiltration with low-viscosity light-curing resins that can rapidly penetrate into the porous enamel. Whereas, S-PRG involves prereacted glass-ionomer technology. This technology forms a stable glass-ionomer phase in fillers by pre-reacting acid-reactive glass containing fluoride with polyacrylic acid in the presence of water.

As there is paucity of information regarding comparison among these remineralizing agent. Hence, this in-vitro study was done to evaluate the remineralizing potential of CPP-ACP with Flouride, Icon and S-PRG fillers, in extracted human permanent teeth using atomic absorption spectroscopy and calorimetric method.

MATERIALS AND METHODS
The present in-vitro study was conducted in the Department of Paedodontics and Preventive Dentistry of CPGIDS&H, Lucknow. Sound premolars, indicated for orthodontic extractions were included in the study. Teeth were excluded if it had- Caries, Morphological variations, fractured crowns, fluorosis and hypoplastic lesion. A total of 60 (sixty) sample teeth were collected. The soft tissue deposits and calculus were removed from the teeth with a surface scaler. The crowns were resected from the roots. Sample teeth were coated with a nail varnish (Colorama nail varnish, Maybelline), leaving a 4 × 4 mm² window on the buccal surface(Fig 1). These 60 teeth samples were divided into three groups, namely Group A, B and C, consisting of 20 sample each. Each sample was immersed in demineralizing solution (composed of 1050 ml of distilled water, 2 g of calcium chloride, 2.2 g potassium hydrogen orthophosphate, 3 g of acetic acid, 56 g of potassium hydroxide) for 4 days to create artificial white spot lesion. The pH of this solution was maintained at 3.5.
After 4 days, the sample teeth were removed from the solution. On the artificially created white spot lesions on teeth samples of Group A, B & C, CPP-ACP with fluoride (GC, MI Varnish, India), Icon (DMG Chemisch-Pharmazeutische Fabrik GmbH, Hambergh, Germany), S-PRG Fillers (Shofu Inc., Kyoto, Japan) was applied as per the manufacturer’s instructions respectively (Fig 2, 3 & 4). Post remineralizing agents application, the samples were immersed in demineralizing solution for 4 days. All the samples were tested for loss of mineral content (wt%) i.e of calcium using atomic absorption spectroscopy and phosphorus using calorimetric method. The loss of ions of all the three groups A, B and C was recorded in microgram/decileter (ug/dl).

The data obtained was tabulated and subjected to statistical analysis. Analysis was performed on SPSS software (Window version 22.0). Data was summarised as Mean ± SE (standard error of the mean). Pre and post groups were compared by paired t test. Pre to post change (pre-post) in outcome measures of three groups were compared by one factor analysis of variance (ANOVA) and the significance of mean difference between (inter) the groups was done by HSD (honestly significant difference) post hoc test after ascertaining normality by and homogeneity of variance between groups by a two-tailed (α=2) P<0.05 was considered statistically significant.

RESULTS
After remineralization, the results showed significant difference between the groups with p-value less than 0.05, when Ca:P were compared, showing greater potential of remineralization for Icon followed by CPP-ACP with fluoride and S-PRG Fillers. In all the three groups i.e Group A(CPP-ACP) with fluoride, Group B (Icon) & Group C (S-PRG Fillers), mean Calcium and Phosphorus ion level decreased after the treatment and this decrease in Calcium and Phosphorus ion level was evidently higher or significant in Group B (Icon) as compared to Group A (CPP-ACP) with fluoride and C (S-PRG Fillers).

Table 1 & 2; Fig 1 & 2

The mean remineralization (i.e mean change from pre test to post test) in Calcium ion of Group B (4.19 ug/dl) was the highest followed by Group A (2.14 ug/dl) and least in Group C (1.8 ug/dl). Whereas, the mean remineralization in Phosphorus ion of Group B (3.29 ug/dl) was the highest followed by Group A (1.73 ug/dl) and least in Group C (1.7 ug/dl). Tukey T test showed significantly different and higher remineralization in Calcium and Phosphorus of Group B (Icon) as compared to both Group A (CPP-ACP) with fluoride and C (S-PRG Fillers). Higher decrease in Calcium and Phosphorus ion loss post treatment denotes that, Group B (Icon) prevents demineralization better. This indicates increased remineralization potential of Icon, followed by CPP-ACP with fluoride and least by S-PRG Fillers.

Figure 1- Samples showing white spot lesions
Figure 2 - Application of CPP-ACP with fluoride (Mfd by GC, MI Varnish, India)

Fig 3 - Application of Icon (Mfd by DMG Chemish-Pharmazeutische Fabrik GmbH, Hambergh, Germany)

Fig 4 - Application of S-PRG Fillers (Mfd by Shofu Inc., Kyoto, Japan)
Table 1: Pre test and post test calcium ion levels (Mean ± SE) of three groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre test (n=20)</th>
<th>Post test (n=20)</th>
<th>Mean change (Pre-Post)</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>45.07 ± 0.73</td>
<td>42.93 ± 0.72</td>
<td>2.14 ± 0.29</td>
<td>7.28</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Group B</td>
<td>45.02 ± 0.80</td>
<td>40.83 ± 0.51</td>
<td>4.19 ± 0.43</td>
<td>9.83</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Group C</td>
<td>46.78 ± 0.58</td>
<td>44.98 ± 0.55</td>
<td>1.81 ± 0.37</td>
<td>4.82</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Table 2: Pre test and post test phosphorus (μg/dl) of three groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre test (n=20)</th>
<th>Post test (n=20)</th>
<th>Mean change (Pre-Post)</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>41.71 ± 0.62</td>
<td>39.98 ± 0.59</td>
<td>1.73 ± 0.13</td>
<td>13.21</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Group B</td>
<td>38.94 ± 0.95</td>
<td>35.66 ± 1.06</td>
<td>3.29 ± 0.32</td>
<td>10.16</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Group C</td>
<td>40.49 ± 0.59</td>
<td>39.33 ± 0.56</td>
<td>1.17 ± 0.12</td>
<td>9.75</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

The pre test and post test phosphorus of three groups were summarised in Mean ± SE and compared by paired t test (t value).

Table 3: Remineralization of calcium (μg/dl) of three groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Remineralization</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>2.14 ± 0.29</td>
<td>12.25</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Group B</td>
<td>4.19 ± 0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>1.81 ± 0.37</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The remineralization of calcium of three groups were summarised in Mean ± SE and compared by ANOVA (F value).

Table 4: Remineralization of phosphorus (μg/dl) of three groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Remineralization</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>1.73 ± 0.13</td>
<td>26.59</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Group B</td>
<td>3.29 ± 0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>1.17 ± 0.12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The remineralization of phosphorus of three groups were summarised in Mean ± SE and compared by ANOVA (F value)

Graph 1. Bar graphs showing comparison of difference in pre test and post test mean calcium of three group.
***p<0.001- as compared to pre test

**Graph 2. Bar graphs showing comparison of difference in pre test and post test mean phosphorus of three groups.**

**Graph 3. Bar graphs showing mean remineralization of calcium of three groups.**

**Graph 4. Bar graphs showing mean remineralization of phosphorus of three groups.**
DISCUSSIONS

Enamel white spot lesions are one of the common problems encountered by the dental practitioner and also a major esthetic concern. The treatment of these white spot lesions should aim to assess the lesion progression and improve the esthetics by eliminating the opacity. Diminishing opacity caused by white spot lesion can be achieved by various non-invasive approaches such as by the use of remineralizing agents. In this regard, fluoride varnishes have been the standard of practice for the professional application of fluoride. Arends & Tencate (1981) observed that salivary remineralization of enamel by topical fluoride has been shown to give rise to predominantly surface remineralization. Thus to achieve substantial remineralization of enamel is a big challenge. The retention of fluoride on enamel and subsurface lesion remineralization depends on the availability of calcium and phosphate ions and combining calcium, phosphate and fluoride ions can lead to loss of bioavailable fluoride ions. To overcome this incompatibility, recently the combination of CPP-ACP with fluoride have been introduced as dental varnish (MI Varnish). Kariya et al demonstrated that when fluoride was added to the CPP-ACP the acid-resisting effect was improved. Ana Coelho et al affirmed that although the remineralization of superficial white spot lesions is often achieved by the application of CPP-ACP, this technique shows unsatisfactory results with respect to old and/ or deep lesions as well as to obtain aesthetics. A recent approach, resin infiltration, infiltration concept (Icon), has evolved in minimally invasive dentistry. It is a microinvasive technique which include resin infiltration up to the depth of the lesion. It was developed at Charite Berlin. A recent invitro study by Subramanian et al showed that this technique is simple, painless, ultraconservative technique that allows immediate treatment of white spot lesions. Newly introduced material S-PRG Fillers offers a more conservative approach as compared to infiltration concept (Icon). These S-PRG Fillers has ability to release and recharge fluoride ions and then it can achieve sustained fluoride release which is acidity dependent. It releases ions like Sr, B, Na and F ions when it comes in contact with water or acidic solution. Hence the present study was conducted to evaluate the remineralizing potential of CPP-ACP with fluoride, Icon and S-PRG Fillers. The pH cycling protocol followed in the present study was as described by Babu et al because this model stimulates the in-vivo caries risk condition. The cycle of demineralization and remineralization was completed by immersing the sample teeth in the demineralizing solution followed by application of remineralizing agent. As observed in the current study, Ali A Assiry have shown in his study the loss of calcium and phosphorus ions when placed in demineralizing solution. In the present study the loss of ions were estimated by atomic absorption spectroscopy and calorimetric method. In the present study, all the three remineralizing agents were able to remineralize the white spot lesions. Group B (Icon) showed the highest remineralizing potential followed by Group A (CPP-ACP) with fluoride and Group C (S-PRG Fillers). It could be because Infiltration concept (Icon) prevents the further progression of initial enamel caries lesion and occludes the microporosities within the lesion. Etching with 15% hydrochloric acid gels leads to a more effective erosion of the surface layer when compared with 37% phosphoric acid gel. Based on such studies, it was shown that resin arrests the progression of white spot by occlusion of the microporosities that provide diffusion pathways for acid and dissolve minerals; it also blocks the further introduction of any nutrients in to the porous system. These findings are similar to the study done by Carlos Rocha Gomes Torres et al who also observed the higher remineralizing potential of resin infiltration(Icon) as compared to CPP-ACP, GC Tooth Mousse, and nano hydroxyapatite (Aclaim) on incipient enamel lesions. Prasad kl et al also observed the higher remineralizing potential of resin infiltration (Icon) as compared to bioactive glass and NaF. Hence, concluded that resin infiltration (Icon) can immediately restore the color of the white spot lesions and stop the progression of emerging caries by blocking the diffusion pathways. In disparity with the present study, Hussain a Batith et al showed that CPP-ACP varnish has higher potential for
remineralizing effect on WSLs of smooth surfaces in comparison to Icon. In tandem to the current study, Wokamatsu et al concluded that the application of PRG coat to WSLs is a more conservative approach as compared to resin infiltration technique. PRG barrier act as an adjunct to periodic fluoride application can promote beneficial remineralisation effect on WSLs.

Higher fluoride concentrations can cause rapid mineral precipitation on the enamel surface and obturation of the surface enamel pores that connect with the underlying demineralized lesion. This process can further limit remineralization of the subsurface demineralized enamel. Jones & Fried reported that an increase in mineral volume from the fluoride-enhanced remineralization can significantly decrease the optical reflectivity of artificial lesions within an enlarged surface zone but not in the subsurface lesion. This could be the reason for the lower level of remineralization in the sample of Group A (CPP-ACP) with fluoride.

The ability of release and recharge of fluoride ions is acidity dependent and under external force these carious lesion treated by S-PRG Fillers may collapse and lead to cavitation. This could be the reason for lowest level of remineralization in the sample of Group C (S-PRG Fillers). To summarize, in the present study we found out that the mean Calcium & Phosphorus ions level decreased after the treatment and the decrease in Calcium & Phosphorus ions level was evidently higher or significant in Group B (Icon) as compared to group A (CPP-ACP) with fluoride and C (S-PRG Fillers). Higher decrease in Calcium & Phosphorus ions loss post treatment denotes that, Group B (Icon) prevents demineralization better. However, there are few limitations in the present in-vitro study, hence further in vivo studies are needed to confirm the results of this study. Based on the observations of this in-vitro study, it can be concluded that Group B (Icon) can be considered as a better remineralizing agent for masking white spot lesion as compared to Group A (CPP-ACP) with Fluoride and Group C (S-PRG Fillers).

REFERENCES:

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