Original Article

# Remineralization Potential Of Pearl Powder Compared To Casein Phosphopeptide Amorphous Calcium Phosphate On Enamel White Spot Lesions (Randomized Clinical Trial)

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## Abstract

**Aim:** The aim of this study was to evaluate the remineralization potential of pearl powder on early ('white spot') lesions in enamel compared to casein phosphopeptide-amorphous calcium phosphate .

**Methodology**: Twenty Patients who had post orthodontic white spot lesions (57 subjects) were included and were randomly allocated in two groups receiving either CPP-ACP as a control (29 subjects) or pearl powder gel (28 subjects), they used it twice daily for three months. The white spot lesions were assessed clinically at baseline, 3,6,9 and 12 months using the Clinical index used for visual evaluation of white spot lesions and photographic image analysis to detect the change in color and area of the white spot lesions. Parametric data were analyzed for intergroup comparisons using independent t-test and for intragroup comparisons using repeated measures ANOVA followed by Bonferroni post hoc test. Non-parametric data were analyzed using Mann-Whitney U test for intergroup comparisons and Friedman's test followed by Nemenyi post hoc test for intragroup comparisons. The significance level was set at $p \leq 0.05$ within all tests.

**Results:** A significant improvement of the clinical WSL-scores was found over time in both groups, with no statistically significant difference ($p<0.01$). As for the photographic image analysis, the color change and area of WSLs significantly improved after12 months with no statistical difference observed between both groups. Conclusions: The Pearl powder had a similar remineralization potential when compared to CPP-ACP on the early enamel white spot lesions after 12 months

**Keywords:** Remineralisation- Post orthodontic white spot lesions- CPP-ACP- Pearl-Randomised clinical trial.

## INTRODUCTION

Dental caries is an infectious microbiologic disease of the teeth that results in localized dissolution and destruction of the calcified tissues. The process of caries formation is a cycle of remineralization and demineralization with various stages being either reversible or irreversible. White spot lesions (WSLs) are manifestations of the earliest stage of caries progression and are capable of being reversible. [1][2]

The development of novel enamel remineralization systems has significantly progressed in recent years with many of them already in clinical use, while others are in various stages of development. The most promising of these remineralizing technologies are the biomimetic regenerative systems. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) complex, a milk protein derivative, has been reported to have anticariogenic effects through buffering of free calcium and phosphate ion activities, by incorporation of ACP into plaque and onto tooth surfaces and thus maintaining a state of super saturation with respect to tooth enamel. [3][4]

However, CPP–ACP is a milk product, it cannot be given to patients having intolerance to milk; therefore a suitable alternative for these patients is required. [5][6]

The pearl and nacre (the mother of pearl) is a naturally occurring composite material composed of inorganic, calcium carbonate plates connected by a framework of organic molecules. Studies, both in vitro and in vivo, have demonstrated nacre's biocompatibility, biodegradability, anti-oxidant and osteogenic potential, which are superior to pure inorganic minerals such as hydroxyapatite or non-degradable metals.[7]

Li et al., 2013 introduced a novel strategy to achieve HAP remineralization of human enamel by using nacre water-soluble matrix as a template for biomimetic remineralization on eroded human enamel. The finding of their study showed that in nacre water-soluble matrix could remineralize the enamel to form similar crystal structure, morphology and lattice orientation to native enamel. While pearl has demonstrated its effectiveness osteogenic, remineralising and anti-oxidant properties, it remains a relatively unexplored biomaterial in the dental field. This current study introduces the clinical usage of pearl as a suggested novel remineralising agent for dental tissue, and discusses the present and future uses of this biologically derived material for application in the dental tissue.

Therefore the aim of the current study was to evaluate the effect of pearl powder on early ('white spot') sub surface lesions in enamel and if it would be capable of promoting remineralization and compare it to Casein phosphopeptide- amorphous calcium phosphate.

## MATERIALS AND METHODS

The protocol of the current study was registered in (www.clinicaltrials.gov) database, with unique identification number (NCT03976583). The study was conducted in Faculty of Dentistry, Cairo University. All procedures performed in this study, involving human participants, were in accordance with the ethical standards of Research Ethics Committee of Faculty of Dentistry, Cairo University (CREC) in July 2019 with ethical approval number (19757). The study design in this investigation was a randomized, controlled, double blinded with two parallel arms. Randomization was obtained with a 1:1 allocation ratio by a computer-generated randomization list (www.random.org).

### Eligibility criteria of participants: [8][9]

Inclusion criteria of participants:

- Males or females.
- Age: 18-40 years old.
- Patients with good oral hygiene.
- Co-operative post orthodontic patients who show interest to participate in the study.

Exclusion criteria of participants:

- Patients with bad oral hygiene.
- Lack of patient's approval and compliance.
- Presence of abnormal oral, medical, or mental condition.
- Patients who are allergic to milk products.
- Pregnant and lactating women.

### Eligibility criteria of teeth:

Inclusion criteria of teeth:

- Obvious area of WSL found on the labial surface of six maxillary anterior teeth that occurred after the orthodontic treatment.

Exclusion criteria of teeth:

- Presence of any restorations including the labial surface of the maxillary six incisor teeth.
- Presence of any environmental or developmental enamel defect such as enamel hypoplasia, fluorosis, stains, and trauma sites on teeth before orthodontic treatment.

### Sample size:

The predicted sample size was a total of (52). Sample size was increased by (20%) to account for possible dropouts during follow-up intervals to be total of (62) cases i.e. (31) for each group.

### Pearl Powder Gel Preparation

Carbopol-940 and sodium CMC were both dispersed in 50ml of distilled water with continuous stirring using magnetic stirrer (Stuart™ hotplate stirrer, SB162). Distilled water was mixed with required quantity of sodium benzoate then heated on water bath to dissolve evenly. Solution was left to cool down and polyethylene glycol-4000 was then added and mixed with the solution. Then required quantity of pearl powder was mixed to the above mixture and volume was making up using remaining distilled water. A flavouring

agent was added to the mix to mimic the CPP-ACP and for better acceptance of the patients. Finally full mixed ingredient were mixed to Carbapol-940 gel in a proper manner with stirring continuously and tri-ethanolamine was then added drop wise to the formulation for adjustment of required pH and to obtain gel in required consistency and concentration 0.6 mg/ml as suggested by Li et al. [10] [11]

## Clinical Procedure:

- The teeth were isolated with sterile cotton roll after its surface was cleaned using sterile gauze pads and polishing with bristle brushes and prophylaxis paste.
- Intraoral standardized digital photographs were taken at baseline and at each recall appointment (0,3,6,9,12 months).
- Modeling of the technique of the application of either treatment to the eligible patients was demonstrated as follows Self-administration was standardized by giving specific instructions to each participant, stipulating that the product should be used twice daily (in the morning and the evening), after a 2 min manual tooth brushing with a fluoride free tooth paste throughout the three months of intervention period. A pea-size amount of the product should be applied per arch, using a dry finger or cotton pellet to distribute it evenly across all teeth and to work it into the interdental spaces. The product was then retained in the mouth for 1–3 min, and manipulated around the teeth using the tongue, before being expectorated. Participants were explicitly instructed not to rinse their mouths after application and not to eat or drink for at least 30 min.[12]. Patients were asked to use a conventional fluoride dentifrice once daily for the entire follow-up period (3-12 months)

## Outcome Measures:

## Visual evaluation of white spot lesions

In the visual method, after thorough drying with air, each lesion around the former bracket base was evaluated at four sites( gingival, mesial, distal and incisal) and graded with respect to WSL severity by a clinical index, the scores and criteria proposed by Andersson where ( 0 scores for no visible color change, 1 scores for slight white color change, only visible after air-drying, 2 scores for slight color change with certain marked white areas , 3 scores for white consistent color change and 4 scores for white consistent color change). [13]

## Standardized Photographic image analysis:

Standardized photographs were taken at 0,3,6,9,12 months. All the photographs were taken with a Nikon D5600 DSLR camera with a 105 mm/2.8 Sigma Macro lens and Meike macro twin flash. The camera was set to manual with an aperture of f22 and a shutter speed of 1/125 of a second. The image quality was set as fine and ISO sensitivity 100. [14]

The change in color was analyzed using Adobe Photoshop CC 2015 (Adobe Systems Inc.). Standardized images were imported to the software and each image was set to the L*a*b* color mode and ΔE was calculated. [15]

$$\Delta E^*_{ab} = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2}$$

As for the change in area analysis for white spot lesions “ImageJ” software version 1.53n for Windows was used, which is an image-processing program that can calculate area and pixel value statistics of user-defined selections. [16].

## Statistical methods:

Categorical data were presented as frequency and percentage values. Numerical data were presented as mean and standard deviation values and were analyzed for normality using Shapiro-Wilk test. Parametric data were analyzed for intergroup comparisons using independent t-test and for intragroup comparisons using repeated measures ANOVA followed by Bonferroni post hoc test. Non-parametric data were analyzed using Mann-Whitney U test for intergroup comparisons and Friedman’s test followed by Nemenyi post hoc test for intragroup comparisons. The significance level was set at $p \leq 0.05$ within all tests. Statistical analysis was performed with R statistical analysis software version 4.1.2 for Windows.

## RESULTS

### 1. Demographic data

This study was conducted on 22 participants with 62 incipient white spot post-orthodontic lesions that were randomly allocated to the intervention and the control arms (n=31), all lesions were found in anterior teeth. After 12 months all participants were assessed with 90% retention rate, as 2 patients failed to attend the whole follow up period. The final patients involved in this study were 20 patients (10 in each group) and white spot lesions involved were 57 lesions simultaneously; (n=29) for the CPP-ACP group and (n=28) for the pearl powder group. There were 16 females (80%) and 4 males (20%) in the current study. Female patients’ percentage in the intervention or control groups was higher than males; 8 females (80 %) and 2 males (20 %) respectively, there was no statistically

significant difference regarding gender among groups (P = 1.0000). (Table 4) Mean age in the current study was 19.9±1.6, in the intervention group mean age was 20.2±1.9, while in the comparator group mean age was 19.6±1.5, there was no statistically significant difference regarding age between groups (P = 0.6487).

## 2. Clinical index used for visual evaluation of white spot lesions

### Descriptive statistics:

There were 116 WSL sites in CPP-ACP group and 112 in pearl powder group. At baseline, majority of CPP-ACP sites 49(42.2%) had score (4) while most of the sites of pearl powder group 70(62.5%) had score (3). After 3 months, majority of CPP-ACP 37(31.9%) and pearl powder 49(43.8%) groups had score (2). After 6 months, majority of CPP-ACP 29(25.0%) and pearl powder 49(43.8%) groups had score (1). After 9 months, majority of CPP-ACP sites 37(31.9%) had score (0) while most of the sites of pearl powder group 51(45.5%) had score (1). After 12 months, majority of CPP-ACP sites 38(32.8%) had score (0) while most of the sites of pearl powder group 50(44.6%) had score (1) as presented in table (1).

**Table (1):** Table showing frequency and percentage values for clinical index score for different groups

| Time | Score | CPP-ACP (n=116) | | Pearl powder (n=112) | |
|---|---|---|---|---|---|
| | | n | % | n | % |
| Baseline | 0 | 0 | 0% | 0 | 0% |
| | 1 | 0 | 0% | 0 | 0% |
| | 2 | 21 | 18.1% | 10 | 8.9% |
| | 3 | 46 | 39.7% | 70 | 62.5% |
| | 4 | 49 | 42.2% | 32 | 28.6% |
| 3 months | 0 | 14 | 12.1% | 7 | 6.2% |
| | 1 | 18 | 15.5% | 31 | 27.7% |
| | 2 | 37 | 31.9% | 49 | 43.8% |
| | 3 | 27 | 23.3% | 19 | 17.0% |
| | 4 | 20 | 17.2% | 6 | 5.4% |
| 6 months | 0 | 27 | 23.3% | 12 | 10.7% |
| | 1 | 29 | 25.0% | 49 | 43.8% |
| | 2 | 23 | 19.8% | 38 | 33.9% |
| | 3 | 20 | 17.2% | 12 | 10.7% |
| | 4 | 17 | 14.7% | 1 | 0.9% |
| 9 months | 0 | 37 | 31.9% | 27 | 24.1% |
| | 1 | 27 | 23.3% | 51 | 45.5% |
| | 2 | 26 | 22.4% | 29 | 25.9% |

| Time | Score | CPP-ACP (n=116) | | Pearl powder (n=112) | |
|---|---|---|---|---|---|
| | | n | % | n | % |
| | **3** | 14 | 12.1% | 4 | 3.6% |
| | **4** | 12 | 10.3% | 1 | 0.9% |
| **12 months** | **0** | 38 | 32.8% | 39 | 34.8% |
| | **1** | 36 | 31.0% | 50 | 44.6% |
| | **2** | 26 | 22.4% | 19 | 17.0% |
| | **3** | 5 | 4.3% | 3 | 2.7% |
| | **4** | 11 | 9.5% | 1 | 0.9% |

## Intergroup and intragroup comparisons:

As a comparison between the two groups regarding the Mean and Standard deviation (SD) values for the visual clinical index score, it revealed that throughout the study at all intervals improvement over time was evident in both groups, however, more sites became invisible in the CPP-ACP group that had a higher mean value than pearl powder group yet the difference was not statistically significant ($p>0.05$)

For the intragroup comparison, there was a remarkable decline in the visual clinical score within each group starting from baseline until 12 months that was statistically significant ($p<0.001$) for both groups. Post hoc pairwise comparisons between the Mean and Standard deviation (SD) values for the visual clinical index score for different intervals showed value measured at baseline to be significantly higher than values measured at other intervals ($p<0.001$). In addition, the value measured after 3 months were found to be significantly higher than values measured at 9 and 12 months ($p<0.001$). Also, they showed value measured after 6 months to be significantly higher than value measured after 12 months ($p<0.001$) as presented in table (2)

**Table (2):** Table showing Mean and Standard deviation (SD) values for clinical index score for different intervals

| Time | Clinical index score (Mean±SD) | | p-value |
|---|---|---|---|
| | **CPP-ACP** | **Pearl powder** | |
| **Baseline** | **3.24±0.66** | **3.20±0.42** | **0.388ns** |
| **3 months** | **2.18±1.03** | **1.88±0.74** | **0.160ns** |
| **6 months** | **1.75±1.11** | **1.47±0.65** | **0.252ns** |
| **9 months** | **1.46±1.08** | **1.12±0.63** | **0.262ns** |
| **12 months** | **1.27±0.96** | **0.90±0.61** | **0.208ns** |
| **p-value** | **<0.001*** | **<0.001*** | |

*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

## 3. Color change

## Intergroup and Intragroup comparisons:

The results comparing the Mean and Standard deviation (SD) values for color change (ΔE) between the two groups showed that pearl powder group had a significantly higher value than CPP-ACP group at 6-9 months and 9-12 months ($p<0.05$). However at other intervals, there was no significant difference between both groups ($p>0.05$)

There was a significant difference between Mean and Standard deviation (SD) values for color change (ΔE) measured at different intervals for both groups ($p<0.001$). Post hoc pairwise comparisons for CPP-ACP group showed value measured at baseline-12 months to be significantly higher than values measured at other intervals ($p<0.001$). In addition, they showed value measured at baseline-3 months to be significantly higher than values measured at 3-6, 6-9, and 9-12 months ($p<0.001$). Post hoc pairwise comparisons for pearl powder group showed values measured at baseline-3 months and baseline-12 months to be significantly higher than values measured at other intervals ($p<0.001$) as presented in table (3).

**Table (3):** Table showing Mean and Standard deviation (SD) values for color change (ΔE) for different groups

| Time | Color change (ΔE) (Mean±SD) | | p-value |
|---|---|---|---|
| | CPP-ACP | Pearl powder | |
| Baseline -3 months | 10.08±2.47 | 9.45±4.57 | 0.518ns |
| 3-6 months | 1.77±0.64 | 2.56±2.00 | 0.058ns |
| 6-9 months | 1.64±0.72 | 2.64±1.00 | <0.001* |
| 9-12 months | 2.12±1.08 | 3.38±1.68 | 0.002* |
| Baseline - 12 months | 11.49±2.80 | 11.37±3.84 | 0.896ns |
| p-value | <0.001* | <0.001* | |

*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

## 4. Area of WSL

### Intergroup and intragroup comparisons:

The Mean and Standard deviation (SD) values for area of WSL for different groups at all intervals showed that CPP-ACP group had a higher value than pearl powder group yet the difference between both groups was not statistically significant ($p>0.05$)

The decline in area of WSL starting from baseline until 12 months was found to be statistically significant for both groups ($p<0.001$). Post hoc pairwise comparisons for the Mean and Standard deviation (SD) values for area of WSL for different intervals showed that CPP-ACP value measured at baseline to be significantly higher than values measured at other intervals ($p<0.001$). In addition, they showed value measured after 3 months to be significantly higher than values measured at 12 months ($p<0.001$). Post hoc pairwise comparisons for pearl powder showed value measured at baseline to be significantly higher than values measured at other intervals ($p<0.001$). In addition, they showed value measured after 3 months to be significantly higher than values measured at 9 and 12 months ($p<0.001$) as presented in table (4).

**Table (4):** Table showing Mean and Standard deviation (SD) values for area of WSL for different groups

| Time | Area of WSL (Mean±SD) | | p-value |
|---|---|---|---|
| | CPP-ACP | Pearl powder | |
| Baseline | 16.90±6.12 | 14.25±5.99 | 0.103ns |
| 3 months | 11.53±3.94 | 10.87±4.59 | 0.563ns |
| 6 months | 10.19±3.51 | 9.90±4.08 | 0.775ns |
| 9 months | 9.67±3.46 | 9.18±3.82 | 0.610ns |

| Time | Area of WSL (Mean±SD) | | p-value |
|---|---|---|---|
| | CPP-ACP | Pearl powder | |
| 12 months | 9.20±3.27 | 8.56±3.85 | 0.501ns |
| p-value | <0.001* | <0.001* | |

*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

## DISCUSSION

White spot lesions, the earliest manifestation of caries progression are categorized as one of the most commonly observed undesirable sequelae associated with fixed orthodontic treatment. The current study there by suggested a new remineralizing agent as the pearl powder to mask the deleterious esthetics of the post-orthodontic WSLs and enhance their remineralization. Furthermore it compared it to the already utilized CPP-ACP as a control.

The CPP-ACP was designated in this study as the comparator as it has demonstrated significantly better remineralizing and anticaries effects compared to a control or fluoride products. Although there is considerable wide variability in the reported results as stated by Philip, 2019. [4]

CPP-ACP is a nano-complex of milk protein that release bioactive agents, that are well known to allow the stabilisation of calcium and phosphate ions resulting in a remarkable supersaturation of soluble ions in saliva for remineralisation of demineralised tooth structure as explained by Fernando et al., 2019. [17]

There is a continuous desire for more natural sources of remineralizing agents that would be both cost effective and economical. As a result, a previous in vitro investigation was performed by Li et al., 2013 to find out a way to take privilege of the numerous advantages of the pearl shell (nacre), as it is considered inexpensive, abundant and safe compared to current materials used for this purpose.[10] Moreover, as compared with shell nacre, pearl contains more organic constituents and trace elements, so it is believed that pearl will behave better than shell nacre as described by Shen et al., 2006. [18]Consequently our research aimed to assess the remineralization potential of pearl powder and to further investigate its efficiency in clinical application.

The pearl powder used in this study is freshwater pearl, from the Zhejiang province in China. The manufacturer Lost Empire Herbs Pearl Powder stated that it is 100% medical grade Pearl Powder (no shell). This pearl powder has 156 mg of highly absorbable calcium per gram besides it is a complete protein, meaning it contains all nine essential amino acids.

Nacre's biocompatibility, biodegradability, and osteogenic potential have been proved in several studies as mentioned by Gerhard et al., 2017. The pearl and nacre (the mother of pearl) is a naturally occurring composite material composed of inorganic, calcium carbonate plates connected by a framework of organic molecules. Similar to mammalian bone, the highly organized microstructure of nacre endows the composite with superior mechanical properties while the organic phase contributes to significant bioactivity that are even superior to pure inorganic minerals such as hydroxyapatite or non-degradable metals. [7]

It is also rich in many other major and trace minerals including magnesium, manganese, strontium and much smaller amounts of many more constituents. There are other amazing compounds found inside of pearls such as Alkaline Phosphatase, which may be one of the active components in biomineralization as stated by Hegde et al., 2014.[19]

The results of our study revealed that pearl powder has succeeded in remineralization of early enamel white spot lesions and enhancing their esthetics. These findings are in accordance with the study of Li et al., 2013 which showed that the enamel remineralized with nacre water-soluble matrix shows great resemblance with similar crystal structure, morphology and lattice orientation to original enamel. Moreover, the Ca/P ratio increased in the presence of nacre; the surface of the remineralized enamel shows a dense and well-aligned layer of HAP coating with homogeneous prism-like crystals densely packed parallel to each other. In addition to this, the remineralized surface had a lower analysis of roughness value and higher Vickers hardness values, which were comparable to those of the original enamel. [10]

The pearl powder used in this study is micronized in which the particle size is 200 mesh or 74 micrometers. However, that might be a limitation of our current study; consequently we recommend further studies to use pearl powder with a nanoparticle size. Nanoparticles have better ion release profiles and capabilities than microparticles. as explained by Arifa et al., 2019.[20]

It's true that the results of our study showed clinical success of the remineralization potential of the pearl powder in treating the initial white spot lesions. However, reliable identification, quantification and characterization of the pearl powder is of great importance to gain a crystal clear understanding of its biochemical mechanism of action and expand it's potential in regenerative dentistry applications.

The assessment methods of the WSLs chosen in the current study were the visual and the photographic image analysis. Gomez et al., 2013 suggested that for both cost and practicality considerations, visual methods should remain the standard for clinical assessment

in dental practice.[21] However, Gomez, 2015 further explained that the diagnosis of initial carious lesions might be more accurately achieved in combination of the visual method and the use of other methods.[22] In addition to this, Singh et al., 2016 recommended that the combined use of technology-based methods and visual assessment should be considered the best approach for evaluating the WSLs.[23] Similarly Hu et al. 2020 stated that individual RCTs should consider assessment of the severity of WSLs using multiple scales and devices rather than single evaluation standards to eliminate the risk of bias and permit combining these elements into meta-analysis.[24]

The visual recordings displayed the interesting finding that both the CPP-ACP protocol as well as the pearl powder seemed to have comparable effects in reducing the WSL scores. This may be a purely optical phenomenon, but as expressed by previous findings by Andersson et al., 2007, a beneficial sub-surface effect of the casein complex is acceptable as illustrated by Reynolds, 1997. [13][25]

Therefore a 'deep' regression with a more favourable aesthetic appearance may be plausible to achieve. Assuming that the perception of WSL is based on its visibility, there was remarkable effect expressed by how many WSL sites disappeared with both treatment groups after 12 months. At baseline, majority of CPP-ACP sites 49(42.2%) had score (4) while most of the sites of pearl powder group 70(62.5%) had score (3). However after 12 months, with a remarkable time positive effect the majority of CPP-ACP sites 38(32.8%) had score (0) while most of the sites of pearl powder group 50(44.6%) had score (1).

In the present study, the treatment for 3 months with both treatments and the follow up for 12 months resulted in significant improvements in change of visual scores compared to baseline. This data was in accordance with the study conducted by Andersson et al., 2007 who found regression in the white spot lesions by 55% in a randomized controlled trial in 26 subjects. [13]Similarly Bailey et al., 2009 who detected regression of 72% after intervention period of 12 weeks. [26]Robertson et al., 2011 in a randomized controlled clinical trial on fifty patients spotted a regression of the WSLs in the MI Paste Plus group by 53.5% compared to the placebo group that increased by 91.1% during the 3 months study period.[8] In addition to this Karabekiroglu et al., 2018 also reported that the daily usage of CPP-ACP was effective in improving the visual appearance of WSLs, and it was found to significantly increase the remineralization of subsurface lesions.[27] On the contrary, Abd ELMoaaty et al., 2021 found that although CPP-ACP had acceptable reminerlaization potential and beneficial effect in masking white spot lesions, however, that improvement regarding the visual analogue scale did not reach to the maximum patient satisfaction in masking white spot lesions. [28] Also, Karabekiroglu et al., 2017 regarded the daily usage of CPP-ACP paste was of no privilege than normal care in improving the appearance of WSLs after 36 months. [29]

To compare between the colour change at base line and throughout the whole follow up period in an objective manor, the difference between two perceived colours in this study is determined by a mathematical equation, in which the colorimetric distance (ΔE) is obtained within the colour space (CIE L*a*b*) appearance. Colorimetric distance is defined as the value that represents the distance between the positions of two colours within the colour space as established by the Comission Internationale de l'Éclairage in 1976. The CIE L*a*b* system is composed of three components: L* denotes the lightness, while a* and b* represents the coordinates on the red–green and yellow–blue color axes, respectively. [30]

The difference in color between the lesions at each time was calculated with the measured CIE L*a*b* values. Kim et al., 2011 explained that ΔE value is a method for quantifying the discrepancy between two colors. [31]They considered ΔE difference of 3.7 units is a clinical indicator for mismatching colours or colour change. That is, white spot lesions are clinically invisible when the ΔE unit between the sound enamel and white spot lesions is of value less than 3.7, and the lesion is clinically visible when ΔE unit between sound enamel and white spot lesions is higher than 3.7. In addition to this Tabatabaian et al., 2021 even stated that the sensitivity of human eyes ($\Delta E < 2.85$). [14]

In the post-stage, after the application of CPP-ACP and pearl powder, it was shown that for both groups a significant decrease in ΔE values measured at different intervals ($p<0.001$) and especially after the 12 months (2.12±1.08) and (3.38±1.68) especially with respect to the pre-stage (10.08±2.47) and (9.45±4.57) for the two groups. These values highly demonstrate the masking effect of both treatments to the appearance of the white spot lesions. Time factor had a positive showed at the values measured at baseline-3 months to be significantly higher than values measured at other intervals ($p<0.001$), which highlight the fact that the treatment protocol was effective as the intervention period was only for the first three months.

Detailed analysis of each of the parameters that constitute the color in the treated WSL area showed that the major decrease in brightness (ΔL) and increased color intensity through the increase (Δa) and (Δb). The remineralization of the white spot lesion for both groups resulted in a noticeable deviation in the patterns of color change with a marked decrease in lightness (L*) and increase in yellowness (b*) and so regaining the color of sound teeth and subsequent loss of the chalky white appearance of white spot lesions which was consistent with the results of Kim et al., 2013.[32] However, Aref and Alrasheed, 2022 found that management of white spot lesions via CPP-ACP solely was not able to restore the esthetic to a non-observable degree of ΔE and suggested combining considerable caries remineralizing programs for more promising results.[33]

Livas et al., 2008 considered that the quantification of the area white spot lesions around orthodontic brackets by means of image analysis software of digitally photographed teeth is a reproducible and accurate method. They further explained that by standardizing the lighting conditions and camera positioning, this method is considered a handy tool for early diagnosis of enamel demineralization. [15]

In the present study both groups showed that there was a decline in area of WSL starting from baseline until 12 months, which was statistically significant ($p<0.001$). Similarly Ebrahimi et al., 2017 found that the application of MI Paste Plus was effective in both

reducing the area as well as increasing the mineral content of WSLs, whereas the control group did not show any significant improvement.[35] Wua et al., 2010 also found that CPP-ACP can reduce the size of demineralized areas of WSLs and promote the remineralization enamel.[36] However, Oliveira et al., 2016 in their in-vitro study found that CPP-ACP products presented similar or even lower performances for lesion area when compared with the control group. They explained that this result was due to several important factors that might have played a role such as the artificial saliva formulation might not reproduce the oral environment precisely.[37]

As our study revealed that after the visual assessment as well as the photographic image analysis of change in color and area of the WSLs, both materials succeeded in promoting the remineralization process with comparable results. Thereby the results of the current study proposed a promising remineralizing agent as the pearl powder to mask the deleterious esthetics of the post-orthodontic WSLs and enhance their remineralization.

## CONCLUSION

1. In highlight of the results of the present study we concluded that The Pearl powder had a similar remineralization potential of the early enamel white spot lesions when compared to CPP-ACP after 12 months
2. . In addition to this, time had a positive effect on the appearance of white spot lesions especially with respect to the pre-stage with no difference between the two materials

## Conflict of interests

There was no conflict of interest for any of the authors


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## REFERENCES


1. Soares R. Assessment of Enamel Remineralisation After Treatment with Four Different Remineralising Agents: A Scanning Electron Microscopy (SEM) Study. J Clin Diagnostic Res. 2017;11(4):136–141.
2. Mittal R, Relhan N, Tangri T. Flourides Casein Phosphopeptide-Amorphous Calcium Fluoride Phosphate Bioactive Glass Casein Phosphopeptide-Amorphous Calcium Phosphate ( CPP-ACP ) Functionalized Tricalcium Phosphate ( fTCP ) Functionalized tricalcium phosphateis produced by milling. Int J Clin Prev Dent. 2017;13(1):1–4.
3. Chiu HF, Hsiao SC, Lu YY, Han YC, Shen YC, Venkatakrishnan K, et al. Efficacy of protein rich pearl powder on antioxidant status in a randomized placebo-controlled trial. J Food Drug Anal. 2018;26(1):309–317.
4. Philip N. State of the Art Enamel Remineralization Systems : The Next Frontier in Caries Management. Caries Res. 2019;284–295.
5. Farooq I, Moheet I, Imran Z, farooq U. A review of novel dental caries preventive material: Casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) complex. King Saud Univ J Dent Sci. 2013;4:47–51.
6. Pithon MM, Baião FS, Sant'Anna LID, Tanaka OM, Cople-Maia L. Effectiveness of casein phosphopeptide-amorphous calcium phosphate-containing products in the prevention and treatment of white spot lesions in orthodontic patients: A systematic review. J Investig Clin Dent. 2019;(July 2018):e12391.
7. Gerhard EM, Wang W, Li C, Guo J, Ozbolat IT, Rahn KM, et al. Design strategies and applications of nacre-based biomaterials. Acta Biomater. 2017;54:21–34.
8. Robertson MA, Kau CH, English JD, Lee RP, Powers J, Nguyen JT. MI Paste Plus to prevent demineralization in orthodontic patients: A prospective randomized controlled trial. Am J Orthod Dentofac Orthop. 2011;140(5):660–668.
9. Heravi F, Ahrari F, Tanbakuchi B. Effectiveness of MI Paste Plus and Remin Pro on remineralization and color improvement of postorthodontic white spot lesions. Dent Res J (Isfahan). 2018;15(2):95–103.
10. Li X, Pan D, Lin S, Zhuang Z, Lin Z. Facile in vitro hydroxyapatite remineralization of human enamel with remarkable hardness. CrystEngComm. 2013;15(21):4351–4356.
11. Telrandhe R, Shende V. FORMULATION AND EVALUATION OF TOOTH GEL FROM ALOE VERA LEAVES EXTRACT INTERNATIONAL JOURNAL OF PHARMACEUTICS & DRUG ANALYSIS Formulation and Evaluation of Tooth Gel from Aloe vera leaves extract. 2017;(October). https://www.researchgate.net/publication/320346541.
12. Llena C, Leyda AM, Forner L. CPP-ACP and CPP-ACFP versus fluoride varnish in remineralisation of early caries lesions. A prospective study. Eur J Paediatr Dent. 2015;16(3):181–186.
13. Andersson A, Anders S, Petersson LG. Calcium Phosphate Complexes on White Spot Lesion Regression Assessed by Laser Fluorescence. Oral Heal Prev Dent. 2007;5(3):229–234.
14. Tabatabaian F, Beyabanaki E, Alirezaei P, Epakchi S. Visual and digital tooth shade selection methods, related effective factors and conditions, and their accuracy and precision: A literature review. J Esthet Restor Dent. 2021;33(8):1084–1104.
15. Tolcachir B, Crohare L, Livas C, Kuijpers-jagtman AM, Bronkhorst E. Quantification of White Spot Lesions around Orthodontic Brackets with Image Analysis. J Oral Res. 2015;78(4):371–377.
16. Hammad SM, Elbanna M, Elzayat I, Mohsen MA. Effect of resin infiltration on white spot lesions after debonding orthodontic brackets. Am J Dent. 2012;25(1):3–8.
17. Fernando JR, Shen P, Sim CPC, Chen YY, Walker GD, Yuan Y, et al. Self-assembly of dental surface nanofilaments and remineralisation by SnF2 and CPP-

ACP nanocomplexes. Sci Rep. 2019;9(1):1–10.
18. Shen Y, Zhu J, Zhang H, Zhao F. In vitro osteogenetic activity of pearl. Biomaterials. 2006;27(2):281–287.
19. Hegde MN, Tahiliani D, Shetty S, Devadiga D. Salivary alkaline phosphatase and calcium in caries-active type II diabetes mellitus patients: An in vivo study. Contemp Clin Dent. 2014;5(4):440–4.
20. Arifa MK, Ephraim R, Rajamani T. Recent Advances in Dental Hard Tissue Remineralization: A Review of Literature. Int J Clin Pediatr Dent. 2019. doi:10.5005/jp-journals-10005-1603.
21. Gomez J, Tellez M, Pretty IA, Ellwood RP, Ismail AI. Non-cavitated carious lesions detection methods: A systematic review. Community Dent Oral Epidemiol. 2013;41(1):55–66.
22. Gomez J. Detection and diagnosis of the early caries lesion. BMC Oral Health. 2015;15(1):1–7.
23. Singh S, Singh SP, Goyal A, Utreja AK, Jena AK. Effects of various remineralizing agents on the outcome of post-orthodontic white spot lesions ( WSLs ): a clinical trial. Prog Orthod. 2016. doi:10.1186/s40510-016-0138-9.
24. Hu H, Feng C, Jiang Z, Wang L, Shrestha S, Yan J, et al. Effectiveness of remineralizing agents in the prevention and reversal of orthodontically induced white spot lesions: a systematic review and network meta-analysis. Clin Oral Investig. 2020;24(12):4153–4167.
25. Reynolds EC. Remineralization of Enamel Subsurface Lesions by Casein Phosphopeptide-stabilized Calcium Phosphate Solutions. J Dent Res. 1997;76(9):1587–1595.
26. Bailey DL, Adams GG, Tsao CE, Hyslop A, Escobar K, Manton DJ, et al. Regression of post-orthodontic lesions by a remineralizing cream. J Dent Res. 2009;88(12):1148–1153.
27. Karabekiroglu S, Aybuke EE, Zehra I, Nimet U. Effectiveness of Remineralization Treatments on Incipient Lesions: A Controlled Clinical Trial. Int J Oral Dent Heal. 2018;4(1):1–9.
28. Abd ELMoaaty D, Mohamed E, Shereen I. TRICALCIUM PHOSPHATE VERSUS CASEIN PHOSPHOPEPTIDE- AMORPHOUS CALCIUM PHOSPHATE IN CARIES WHITE SPOT. Egypt Dent J. 2021;67:2613–2622.
29. Karabekiroğlu S, Ünlü N, Küçükyilmaz E, Şener S, Botsali MS, Malkoç S. Treatment of post-orthodontic white spot lesions with CPP-ACP paste: A three year follow up study. Dent Mater J. 2017;36(6):791–797.
30. International Commission on Illumination., CIE Technical Committee 1-47. Improvement to industrial colour-difference evaluation. 2001;10.
31. Kim S, Kim EY, Jeong TS, Kim JW. The evaluation of resin infiltration for masking labial enamel white spot lesions. Int J Paediatr Dent. 2011;21(4):241–248.
32. Kim Y, Son H, Yi K, Kim H, Ahn J, Chang J. The color change in artificial white spot lesions measured using a spectroradiometer. Clin Oral Investig. 2013;17:139–146.
33. Aref NS, Alrasheed MK. Casein phosphopeptide amorphous calcium phosphate and universal adhesive resin as a complementary approach for management of white spot lesions: an in-vitro study. Prog Orthod. 2022;23(1). doi:10.1186/s40510-022-00404-9.
34. Livas C, Kuijpers-jagtman AM, Bronkhorst E. Quantification of White Spot Lesions around Orthodontic Brackets with Image Analysis. Angle Orthod. 2008;78(4):585–590.
35. Ebrahimi M, Mehrabkhani M, Ahrari F, Parisay I, Jahantigh M. The effects of three remineralizing agents on regression of white spot lesions in children: A two-week, single-blind, randomized clinical trial. J Clin Exp Dent. 2017;9(5):e641–e648.
36. Wua G, Liub X, Houc Y. Analysis of the effect of CPP-ACP tooth mousse on enamel remineralization by circularly polarized images. Angle Orthod. 2010;80(5):933–938.
37. Oliveira G, Ritter A V., Heymann HO, Jr. ES, Donovan T, Guy Brock. Remineralization effect of cpp-Acp and fluoride for white spote lesion in vitro. J Dent. 2016;42(12):95–121.