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Original Article

Remineralizing Effect and Ion Release of Pearl Powder versus Casein Phosphopeptide Amorphous Calcium Phosphate on Non-cavitated Initial Enamel Lesions: An In Vitro Study

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Abstract

Aim: To investigate the remineralization potential and ion release of pearl powder versus casein phosphopeptide amorphous calcium phosphate paste (CPP-ACP) on artificial initial enamel lesions using laser fluorescence analysis. **Material and Methods:** Twenty-four extracted sound human molars were divided randomly into two groups (n=12) based on the remineralizing agent, Group 1: pearl powder gel and Group 2: CPP-ACP paste. Quantitative measurements using DIAGNOdent pen were performed at baseline, after demineralization, after two weeks and four weeks remineralization. The ion release of each remineralizing agent was assessed to detect different released analytes. Data was statistically analyzed with significance level ($P \leq 0.05$) where intergroup comparison using independent t test and intragroup comparison using repeated measures ANOVA followed by Bonferroni correction was performed. **Results:** No statistically significant difference was observed between both groups at baseline, after demineralization and two weeks remineralization ($P > 0.05$) while a significant difference was found after four weeks remineralization ($P = 0.0137$). Intragroup comparison revealed a significant difference with time ($P < 0.001$). Ion release revealed that calcium, sodium and strontium analytes were detected in both groups. **Conclusion:** Pearl powder demonstrated comparable remineralization potential to CPP-ACP, acting as a promising regimen in treating initial enamel lesions. Additionally, repeated application helped gain more remineralizing results from the applied agents.

Keywords: Pearl powder, CPP-ACP, Remineralization, Ion Release, Initial enamel lesions

I. INTRODUCTION

Dental caries develops when an imbalance in the demineralization and remineralization process occurs which is influenced by dietary factors, host conditions, and acid-producing bacteria ***(Ingale et al., 2023)***. Demineralization leads to mineral loss due to the dissolution of hydroxyapatite, creating porosities within the hard dental tissue ***(Lopes et al., 2024)***.

The latest approach to caries management focuses on detecting early non-cavitated enamel lesions promptly and treating them non-invasively. These early carious lesions can be reversed; the partially demineralized hydroxyapatite crystals can return to their original size when exposed to oral conditions rich in remineralizing substances ***(Eliwa et al., 2022)***.

Remineralization is a natural repair process utilizing calcium and phosphate ions along with fluoride, to recreate a new surface on the remaining crystals of demineralized subsurface lesions

(Hamba et al., 2020). While fluoride is widely used, its limitation of inducing surface remineralization at the expense of the lesion body complicates the process of achieving complete remineralization. This has led to the development of calcium phosphate systems, including casein phosphopeptide amorphous calcium phosphate, bioactive glass and functionalized tricalcium phosphate ***(Arjun et al., 2021)***.

Casein phosphopeptide amorphous calcium phosphate (CPP-ACP) complex, derived from milk proteins, has been shown to provide free calcium and phosphate ions. With the presence of CPP, stabilizing high concentration of ACP is possible, serving as a reservoir for calcium and phosphate ions. It incorporates amorphous calcium phosphate into dental plaque and onto tooth surfaces, helping to maintain a state of supersaturation aiding remineralization ***(El-sherif et al., 2022)***.

The development of novel and effective approaches to enhance the remineralization of early enamel lesions is still ongoing. One promising material is pearl powder ***(El Damarisy et al., 2022)***. Pearl powder is well-regarded in traditional Chinese medicine for a wide range of uses. Its application is increasingly expanding in the biomedical field, where benefits have been attributed to the active components within the pearl matrix. Pearl powder has been used to treat bone-related conditions due to its high content of proteins and minerals, contributing to its strong osteogenic properties ***(Jun Loh et al., 2021)***. Calcium carbonate ($CaCO_3$), the primary chemical component of pearl, serves as a natural source of calcium together with other elements available like amino acids, zinc, magnesium, sodium, and iron ***(Aidaros and Kamh, 2021)***.

Due to the limited available literature regarding the remineralizing efficacy of pearl powder, this study was conducted to investigate the remineralization potential of pearl powder compared to CPP-ACP on initial enamel lesions. The null hypothesis tested was that both materials would show similar remineralization results on initial enamel lesions.

II. MATERIALS AND METHODS

A. Materials

The materials used in this invitro study were:

- *Casein phosphopeptide amorphous calcium phosphate (GC Tooth Mousse®, GC America Inc, Illinois, USA)*

A crème containing 10% Casein phosphopeptide amorphous calcium phosphate (CPP-ACP), a milk derivative phosphorprotein known to have the ability to bind and stabilize calcium and phosphate in solution, as well as to bind to dental plaque and tooth enamel.

- *Pearl Powder (Lost Empire Herbs, Missouri, USA)*

A powder derived from Hyriopsis cumingii, a type of freshwater pearl, which is mineral rich with calcium being the major mineral it contains. It is also rich in many other elements including magnesium, manganese, strontium. Pearl powder is a complete protein, as it contains all nine essential amino acids, including Aspartic acid, Lysine, Alanine, Arginine, Serine, Phenylalanine, Methionine, Glutamic acid, Glycine, Leucine, Proline, Tyrosine and Cystine. It helps with bone mineralization and regulation of osteoblast and osteoclast function.

B. Methods

- **Sample Size Calculation**

In a previous study by ***Arjun et al., 2021***, the DIAGNOdent score within CPP ACP group after 30 days was normally distributed with standard deviation 1.28. If the true difference between pearl powder and CPP ACP means is 1.5, we will need to study 12 teeth per group to reject the null hypothesis that the experimental and control groups' population means are equal with a probability (power) of 0.8. Type I error probability associated with this test of this null hypothesis is 0.05. Sample size was estimated using PS Power and Sample for Windows version 3.1.6 using independent t test.

- **Preparation of Pearl Powder Gel**

The preparation of the pearl powder involved dispersing Carbopol-940 and sodium CMC in 50 milliliters of distilled water while continuously stirring with a magnetic stirrer (MMS-3000, Biosan Ltd, Riga, Latvia). Five milliliters of distilled water was combined with the necessary amount of sodium benzoate and heated in a water bath until fully dissolved. After cooling, polyethylene glycol 4000 was added. Then, 4.8 grams of pearl powder were accurately measured and incorporated into the mixture. The total volume is adjusted with the remaining distilled water to achieve a concentration of 0.6 mg/mL-1, as recommended by ***Li et al., 2013***.

Carbopol-940 is then added to ensure proper gelling while stirring continuously, and tri-ethanolamine is gradually added to adjust the pH and achieve the desired consistency. The amounts of Carbopol and sodium CMC were varied to modify homogeneity, viscosity and spreadability. Finally, the mixture was stored in a refrigerator at 8°C for 24 hours before further use ***(El Damarisy et al., 2022)***.

- **Specimen Preparation**

Twenty-four freshly extracted permanent, sound human molars were collected from the Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Cairo University. Teeth were cleaned from any evident blood and debris, then autoclaved for 40 minutes to inhibit microbial growth ***(Shetty et al., 2014)***. The teeth were cleaned with ultrasonic tips and rubber cup/pumice prophylaxis, then stored in a thymol solution for two weeks. The roots were trimmed 2mm below the cementoenamel junction using a microtome (Leica 1600 saw microtome, Wetzlar, Germany). Afterwards, they were checked under a stereomicroscope (Leica S8 APO, Wetzlar, Germany) at ×40 magnification to ensure no enamel defects, cracks or caries were detected. Each tooth crown was inserted in a self-cured acrylic resin mold with the buccal surface positioned upwards. The buccal surface was double coated with acid-resistant nail varnish, leaving four exposed enamel windows, each measured 2mm × 2mm. The first window was painted with nail varnish, acting as a control for baseline ***(Kamal et al., 2020)***.

- **Artificial Initial Enamel Lesion Formation**

Each specimen was individually immersed in a daily refreshed 15ml demineralizing solution, for three consecutive days (72 hours) at 37°C in an incubator to develop a uniform white spot lesion. The demineralizing solution was a carbonated beverage (Pepsi, PepsiCo Inc., New Bern, North Carolina) of pH 1.28 (carbonated water, fructose, phosphoric acid, caffeine, citric acid) ***(Eliwa et al., 2022)***. Afterward, the specimens were rinsed thoroughly and stored in distilled deionized water. Th second window was painted with nail varnish as a control for demineralization.

- **Remineralizing Agents Application**

Specimens were randomly assigned to two groups (n=12) according to the remineralization regimen, pearl powder group (intervention) and CPP-ACP group (comparator). To standardize the application method, each material was applied on the lesion surface using a cotton-tip applicator, rubbed in a circular motion for three minutes. Subsequently, excess was gently wiped off with a cotton-tip applicator so that any material residue would gradually disperse when stored in artificial saliva, which simulates clinical oral application. The artificial saliva contained 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4 and 0.15 mM potassium chloride at pH 7.0. Each specimen was immersed individually in a daily renewed artificial saliva ***(Kamal et al., 2020)***.

After two weeks of remineralization, the third window was covered with nail varnish. Following four weeks of remineralization, the nail varnish was gently removed, and the specimens were subjected to quantitative laser fluorescence assessment.

- **Quantitative Laser Fluorescence Analysis**

Quantitative readings were obtained using a laser fluorescence device (DIAGNOdent pen 2190, KaVo Dental, Biberach an der Riss, Germany). Before each reading, the device was calibrated using its own ceramic disk. Specimens were positioned horizontally with the lesion facing upward, ensuring standardization by holding the tip at a 90° angle to the lesion surface. Readings were interpreted according to DIAGNOdent criteria where 0-7 indicates healthy tooth structure, 8-15 indicates initial demineralization, and values >16 indicates significant demineralization. ***(Arjun et al., 2021; Eliwa et al., 2022)***. Specimens were assessed at baseline, after demineralization, and following remineralization at two weeks and four weeks intervals.

- **Ion Release Analysis**

The release of calcium, phosphorous, sodium and strontium were analyzed. Each material was mixed with deionized water at a 1 mg/ml ratio, and the mixture was stirred for one hour with a magnetic stirrer until fully dispersed. Next, the mixture was centrifuged for 30 minutes to obtain clear supernatants. The ion-release was quantified from the supernatants using a triple-quadrupole inductively coupled plasma-mass spectrometry (ICP-MS/MS) instrument (ICP-QQQ, Agilent 8800, Agilent Technologies, California, USA). The released ions are expressed in milligrams per gram (mg/g) unit ***(Shaalan and El-Rashidy, 2023).***

- **Statistical Analysis**

Data were analyzed using Medcalc software, version 22 for Windows (MedCalc Software Ltd,

Ostend, Belgium). The normality of continuous data was assessed using Kolmogorov-Smirnov test and Shapiro-Wilk test. Continuous data showed normal distribution and were described using mean and standard deviation. Intergroup comparison of DIAGNOdent readings was carried out with independent t test. Intragroup comparison was carried out using repeated measures ANOVA followed by Bonferroni corrected pairwise comparisons. Statistical power was set at 80 % with 95 % confidence level and all tests were two tailed, with statistical significance set at ($P \leq 0.05$).

III. RESULTS

A. Quantitative Laser Fluorescence Results

A significant reduction in laser fluorescence readings was observed after two weeks, continuing to progressively decrease after four weeks.

Intergroup comparison showed no significant difference at baseline, demineralization and after two weeks of remineralization ($P>0.05$), indicating both materials notably promoted enamel remineralization. However, after four weeks remineralization, a significant difference was detected between the two groups ($P=0.0137$), favoring CPP-ACP in its higher remineralization potential. Intragroup comparison within each group revealed a statistically significant difference over time ($P<0.001$), revealing that the tested materials yielded more effective remineralization results with extended application times. The mean and standard deviation values of the two groups at different testing intervals are shown in table (1) and figure (1).

B. Ion Release Results

The ion release analysis revealed that calcium, sodium and strontium were detected in pearl powder while calcium, phosphorus, sodium and strontium were detected in CPP-ACP. Both materials showed calcium as the most abundant, indicating its role in the remineralization process. The analytes detected in both materials are presented in table (2) and figure (2).

Table (1): Mean and standard deviation of laser fluorescence scores of tested groups at each time interval

Material / Follow-up	Pearl Powder		CPP-ACP		Difference	95% CI	P value
	Mean	SD	Mean	SD			
Baseline	0.75^{a}	0.75	0.58^{a}	0.66	-0.16	-0.769 to 0.436	0.5724
Demineralization	18.4^{c}	6.52	22.66^{c}	4.67	4.25	-0.558 to 9.058	0.0804
2 weeks Remineralization	6.16^{b}	2.75	6.00^{b}	1.04	-0.16	-1.932 to 1.598	0.8466
4 weeks Remineralization	4.33^{b}	1.07	5.41^{b}	0.90	1.08	0.244 to 1.921	0.0137*
P value	<0.001*		<0.001*				

*Means that do not share a letter are statistically significant vertically, *corresponds to statistically significant*

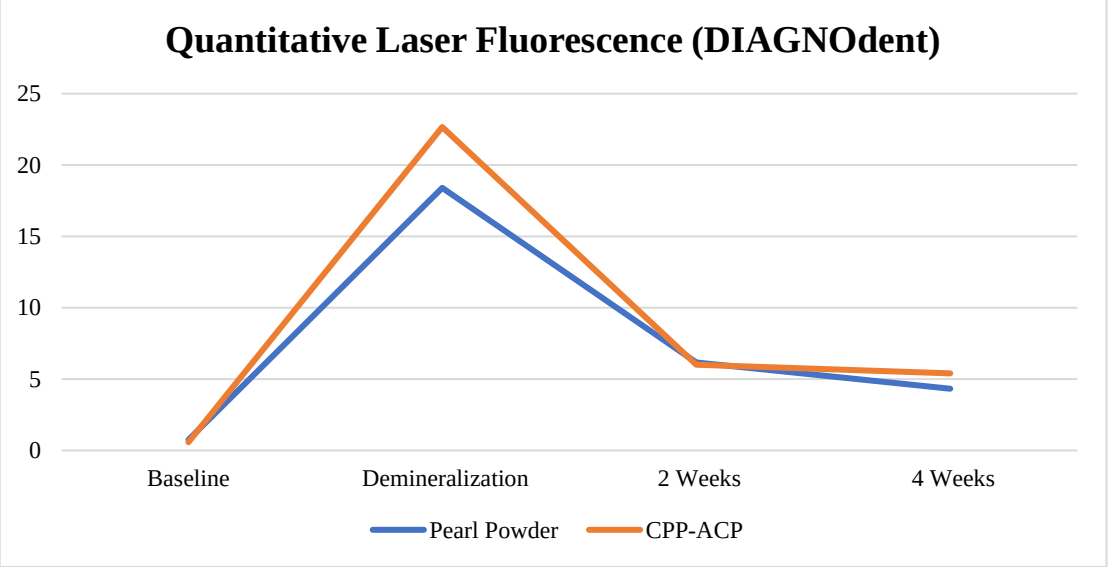

Figure (1): Graph illustrating laser fluorescence scores of tested groups at each time interval

Table (2): Different Analytes of ion release results of pearl powder and CPP-ACP group

Analyte (mg/g)	Pearl Powder	CPP-ACP
Calcium	16.885	9.568
Phosphorus	ND	6.530
Sodium	2.214	5.306
Strontium	0.1482	0.006

ND: Not detectable

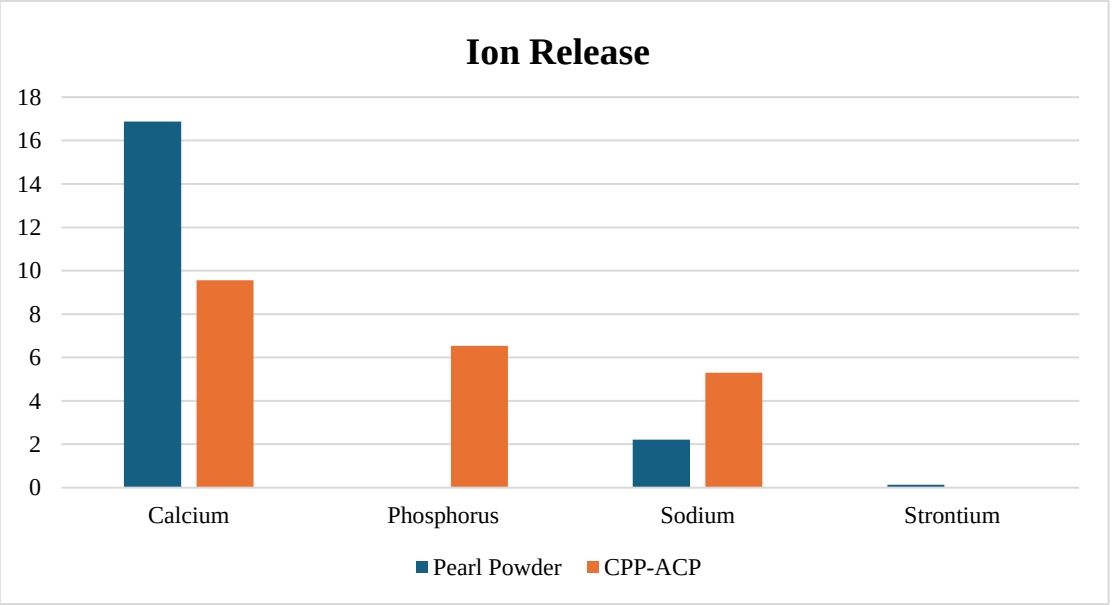

Figure (1): Graph illustrating ion release analytes of tested groups

IV. DISCUSSION

Minimal invasive dentistry entails preserving dental tissue, either by caries prevention or reversing initial lesions. Early, non-cavitated lesions are treated non-invasively with therapeutic remineralizing agents. There is a growing demand for safer, natural agents, such as pearl and seashell powder, that might offer enhanced remineralization efficiency ***(Desai et al., 2021)***.

Pearl powder has been utilized for thousands of years in medicine, cosmetics, and as a health food supplement. It is rich in proteins and minerals and has been traditionally used to treat various skin and bone conditions ***(Loh et al., 2021)***. Several studies have reported pearl seashell powders are biocompatible and non-toxic ***(Brundavanam et al., 2017; Aidaros and Kamh, 2021).***

The significance of pearl powder as a natural source of calcium has been well-established, leading to increased interest in its application in the medical field. As a result of being safe, abundant and inexpensive, researchers have focused on exploring its potential uses. It is proposed that pearl powder possesses properties similar to bone, and exhibits osteoconductive, osteoinductive, and osteogenic properties ***(Eliwa et al., 2022)***. Pearl powder is primarily composed of calcium carbonate ($CaCO_3$), with an organic matrix consisting of proteins, glycoproteins, and polysaccharides. These components were found to stimulate bone formation in vitro and in vivo and are believed to support tooth remineralization. It also contains other trace elements such as sodium, manganese, selenium, aluminum, and strontium ***(El Damarisy et al., 2022)***.

Casein phosphopeptide amorphous calcium phosphate (CPP-ACP) was used in this study as it is considered the gold standard for non-fluoridated remineralizing agents ***(Arjun et al., 2021)*** and testified with the most promising clinical results ***(El Damarisy et al., 2022)***. Remineraliztion of CPP-ACP relies on the presence of multiphosphoseryl-containing sequences in the compound. CPP helps stabilize high concentrations of amorphous calcium phosphate (ACP), acting as a calcium reservoir. Additionally, CPP-ACP is reported to neutralize mouth acids effectively ***(Ingale et al., 2023)***.

Traditionally, the diagnosis of initial enamel lesions relied on visual examinations to assess the

extent and depth of the lesions. However, new techniques such as fluorescence analysis have emerged. Originally used in vivo, fluorescence analysis is now employed as a research tool for in vitro studies. The goal is to reach a more accurate lesion diagnosis, with better insights regarding its depth and extent. This, in turn, helps improve treatment and prognosis ***(Lopes et al., 2024)***. Accordingly, quantitative laser fluorescence analysis using DIAGNOdent was used as a non-invasive diagnostic tool to assess the demineralized enamel lesions and monitor the remineralization progress during the study follow-up. DIAGNOdent is known to detect early enamel lesions and evaluate the effectiveness of the applied interventions due to its high sensitivity. Various studies highlighted the reliability of DIAGNOdent as a dependable non-invasive early caries detection device ***(Eliwa et al., 2022)***.

This study results revealed that both groups showed a significant reduction in the quantitative laser fluorescence readings with time ($P<0.001$) where the lowest scores were achieved after 30 days remineralization. This denotes the amount of remineralization is dose dependent and increases with repeated application of the used agents. This is in accordance with many studies confirming that remineralization is a time dependent process ***(Oliveira et al., 2016; Kamal et al., 2018; Esparza-Villalpando et al., 2021; Alkarad et al., 2023)***.

For intergroup comparison, no statistically significant difference was observed ($P>0.05$) at baseline, demineralization and after two weeks remineralization. However, after four weeks, a statistically significant difference ($P=0.0137$) was observed where CPP-ACP group showed higher remineralization potential compared to the pearl powder group.

The higher remineralizing efficiency of CPP-ACP is attributed to its copious amounts of available calcium and phosphate ions. CPP-ACP is a bioactive compound formed of two components: casein phosphopeptides (CPPs) and amorphous calcium phosphate (ACP). CPP, a phosphoprotein derived from milk, has a unique ability to stabilize high concentrations of ACP clusters, inhibiting them from growing to the necessary size for nucleation and precipitation. When applied, CPP-ACP nanocomplexes adhere to tooth surface and dental plaque, creating a reservoir of loosely bound calcium and phosphate ions, which would penetrate the lesion body, deposit into the crystal voids, aiding in remineralization. The results agree with serval studies confirming the remineralization potential of CPP-ACP ***(Imani et al., 2019; Kamal et al., 2020; Cardoso-Martins et al., 2022; Hadidi et al., 2022).***

The enamel remineralization ability of the pearl group is due to the high amount of calcium available, credited to its rich calcium carbonate ($CaCO_3$) content. Research clarified that the water-soluble matrix in pearl functions as a natural template for $CaCO_3$, serving as claws to capture calcium ions and arrange them, thereby promoting hydroxyapatite nucleation on the enamel surface ***(El Damarisy et al., 2022)***. Additionally, it is believed these complex proteins and organic macromolecules serve as a binder, essential for the creation of densely packed hydroxyapatite nanorods, which contribute to a hardness level comparable to natural enamel. It was found that the enamel remineralized with pearl closely resembled the original enamel, exhibiting similar crystal structure, morphology, and lattice orientation. ***(Li et al., 2013; El-Sherif et al., 2022)***.

$CaCO_3$ is pH-sensitive, and low acidic pH can trigger its dissolution. This may explain the release and leaching out of Ca ions. Thus, $CaCO_3$ serves as a calcium reservoir, enabling the release of Ca ions during acidic challenges, increasing Ca ions concentration in saliva. This elevated calcium in saliva supports remineralization. Additionally, the released carbonate (CO3) ions act as a buffering agent in saliva ***(Aidaros and Kamh, 2021).***

The study findings align with the work of ***Aidaros and Kamh, 2021*** and ***Eliwa et al., 2022*** who confirmed that the use of pearl resulted in significant enamel mineral gain and improved surface microhardness. They described the composition of pearl to be a crystalline $CaCO_3$ mineral phase embedded in an organic matrix. The crystals are encased by an organic layer of proteins and polysaccharides, including Perlucin, Lustrin A, and Perlustrin. Studies have shown that Perlucin plays a key role in facilitating biomineralization, as well as promoting the nucleation and orientation of $CaCO_3$ crystals ***(Mailafiya et al., 2019)***.

Other studies by ***El Damarisy et al., 2022*** and ***El-Sherif et al., 2022*** evaluated the effect of pearl and found it to be successful in remineralizing enamel white spot lesions while improving their esthetics. It was noted that pearl composition closely

resembles that of human enamel, consisting of over 95 wt.% inorganic material and 1-5 wt.% organic matter. The soluble matrix was shown to promote highly ordered crystallization within the pearl structure, and it was reported to encourage the growth of hydroxyapatite crystals. They concluded that pearl offers a novel, non-invasive, approach for treating early, non-cavitated enamel lesions.

Regarding ion release, calcium was detected in both groups, while phosphorus was detected only in the CPP-ACP group. This study results are in agreement with studies done by ***Aidaros and Kamh, 2021*** and ***Eliwa et al., 2022*** where they also detected a higher concentration of Ca ions in the nano-pearl group. They justified the greater release of calcium ions contributed to the biomimetic remineralization of initial enamel lesions by forming crystal nuclei and promoting crystal deposition and growth.

Detection of sodium and strontium in both groups is of great importance in the dental field. Sodium plays an indirect yet vital role in maintaining a stable pH in the oral cavity by supporting the buffering capacity of saliva, thereby reducing the harmful effects of acids on hard dental tissue. Maintaining a balanced ionic environment through adequate sodium concentration in saliva is essential for optimal oral health and prevention of dental caries. Sodium ions (Na) help maintain the osmotic balance of saliva and assist in transporting bicarbonate ions. They help ensure the bicarbonate ions (HCO_3) are present at sufficient concentrations to neutralize acids. Sodium is important for the solubility of bicarbonate in the oral environment, ensuring that bicarbonate is available to buffer excess hydrogen ions ***(Murayama et al., 2018; Poletto et al., 2022)***.

Strontium is often included in dental remineralizing agents. Strontium has similar ionic properties to calcium, which is a critical component of hydroxyapatite. When strontium ions are applied to demineralized enamel, they can replace some of the calcium in the enamel structure, leading to the formation of strontium hydroxyapatite. This process can strengthen enamel by enhancing its mineral content and reduce the risk dental caries by preventing further demineralization ***(Dotta et al., 2022)***. Strontium's oral bioavailability means that when it is ingested in small amounts (through supplements or foods), it can be absorbed and transported to areas of the body, including the teeth and bones. This systemic effect could potentially help support overall bone health, which, in turn, supports the structural integrity of teeth, reduce the risk of bone loss, especially in individuals with periodontitis or osteoporosis, potentially enhancing tooth retention and stability ***(Mathew et al., 2018; Dotta et al., 2022; Rajendran et al., 2024)***.

The limitations of the current study include the micronized pearl powder (74 micrometers) which may have influenced its bioavailability, solubility and deposition. Accordingly, the use of nano-sized pearl powder might offer enhanced ion release and remineralization ability. Further studies are advised to confirm these findings and determine the proper regimen of pearl powder in terms of formulation, delivery vehicle, dose and application period to achieve optimum remineralization.

V. CONCLUSION

Under the limitations of this study, it can be concluded that pearl powder exhibited comparable remineralization potential to CPP-ACP, serving as a promising treatment option for early, non-cavitated enamel lesions. Moreover, frequent application improves the remineralization effect of these agents.

Conflict of Interest:

The authors declare no conflict of interest.

Funding:

This research received no funding grant from any agency in the commercial, not-for-profit or public sectors.

Ethics:

The study received approval by the research ethics committee (REC), Faculty of Dentistry, Cairo University on 26-3-2024, with Approval ID: 31324.

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