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Comparative Evaluation Of Changes In CA, P And F Levels After Creating An Artificial White Spot Lesion And Subjecting Them To Different Remineralizing Agents: An Edax Analysis

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Abstract:
Background*: With rising demand for esthetic restorations, shade matching in dentistry blends art and science-affected by several external factors. While visual shade selection with a guide remains common for its simplicity, newer technologies like spectrophotometers and digital photography offer more objective, detailed color measurement and improved communication.*
Aim*: The Aim of the study is to compare and evaluate the remineralizing ability of five different remineralizing agents on artificially created white spot lesions.*
Materials and Methods*: Fifty maxillary premolars extracted for orthodontic purposes were taken for the study. Teeth are cleaned using ultrasonics and stored in 0.1 % thymol solution. 4 × 4 mm label placed on buccal surface of the crown. Remaining portion of tooth was painted with nail varnish. The samples were subjected to a demineralizing solution (0.264 gm of monosodium phosphate,0.244 gm of calcium chloride, 2.86 ml of acetic acid in 1l of deionised water) for 4 days to create an artificial white spot lesion. The samples are divided into 5 groups based on the remineralizing agent used. Group I : CPP-ACP; group 2: egg shell solution; group 3: red marine algae; group 4: bioactive glass nanoparticle solution; group 5: acclaim toothpaste. All the samples are sectioned to obtain the buccal cusp containing the experiment window and the EDAX analysis performed to obtain the ca, p and F values. The samples will be immersed in respective remineralising agents for 5 minutes, twice daily for a period of 6days and subjected to EDAX to obtain the ca p and f values after remineralization. The Ca, P and F values obtained after demineralization and remineralization will be compared.*
Results*: Bioactive glass nanoparticle solution showed higher amount of remineralization compared to all the other study groups. The Ca, P and F values indicates the remineralization potential of all the study groups.*
Conclusion: *Within the limits; the present study concluded that the Ca, P and F values of Bioactive glass nanoparticle solution have been increased significantly followed by Acclaim tooth paste, CPP ACP, Red marine algae and Egg shell solution*
Key Word*: Bioactive glass nanoparticle, CPP ACP, Egg shell solution, Red marine algae and Acclaim tooth paste.*

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I. Introduction

The current trend in caries management is early detection and targeted non-management of invasive reversible lesions using novel remineralization agents. Chemical demineralization of teeth is caused by acidic attack through two primary means: dietary acid consumed through food or drink/drugs and microbial attack from bacteria present in the mouth. During an acidic attack, or a typical demineralization regime, chemical dissolution of both the organic and inorganic matrix components takes place. This is brought about by the water content of

enamel and dentine, which facilitate acid diffusion in and mineral content out of tooth. Initial demineralization is a reversible process; hence, the partially demineralized hydroxyapatite (HA) crystals in teeth can grow to their original size if they are exposed to oral environments that favor remineralization. Dental caries can be arrested or repaired by enhancing teeth mineralization at early stages.

Role of fluoride in remineralization is long known and time tested to be the effective method of remineralization. With the advancement in science and research, newer remineralising agents such as CPP ACP, egg shell solution, bioactive glass nanoparticle solution, red marine algae and acclaim tooth paste has been tested in this study. With the advent of all these systems there is great need to quantitatively evaluate the amount of remineralization and one of the techniques to assess the changes in tooth's mineral content is Scanning Electron Microscope (SEM) equipped with EDAX micro analyser

In the present study, the biotechnological potential of the remaining skeleton of the macroalgae lithothamnion calcareum (LC) was tested in its ability to modulate the enamel de-remineralizing dynamics. LC is a red macroalgae of the corallinaceae family, which is abundantly found on the Brazilian coastline. A unique feature of LC is the presence of calcium and magnesium carbonate precipitates in its cell walls and enamel mineralization mainly lies in its high calcium content. Based on the principle that enamel remineralization can be enhanced when it gets exposed to saturated calcium delivering sources.

Early intervention on white spot enamel lesion is possible with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) complex, a milk protein derivative, that has been proven to have anticariogenic effects. It prevents enamel demineralization and promotes remineralization through buffering free calcium and phosphate ion activities, thereby incorporating ACP into plaque and onto tooth surface and maintaining a state of supersaturation with respect to tooth enamel. Remineralized enamel has an increased resistance to subsequent acid challenge than untreated enamel.

Eggshell powder (ESP) is well known for being a rich source of calcium and essential elements. It contains about 1% calcium phosphate, 94% calcium carbonate, and 1% magnesium carbonate. Thus, ESP was proven to be efficient in remineralization of the enamel surface, especially when it is used as a solution since its minerals could easily diffuse into the enamel obstructing the enamel surface porosities.

Nano-hydroxyapatite (nHA) is a biocompatible and bioactive agent. It attracts large amounts of calcium and phosphorous ions from the remineralizing solutions to the enamel surface promoting enamel remineralization. BAG is a ceramic material consisting of amorphous sodium-calcium-phosphosilicate which is highly reactive in water and as a fine particle size powder can physically occlude dentinal tubules. In the aqueous environment around the tooth, i.e., saliva in the oral cavity, sodium ions from the BAG particles rapidly exchange with hydrogen cations (in the form of H_3O^+) and this brings about the release of calcium and phosphate ($PO4^-$) ions from the glass. A localized, transient increase in pH occurs during the initial exposure of the material to water due to the release of sodium. This increase in pH helps to precipitate the extra calcium and phosphate ions provided by the BAG to form a calcium phosphate layer. As these reactions continue, this layer crystallizes into hydroxycarbonate apatite

II. Material And Methods

This invitro comparative study was carried out in the Department of Conservative Dentistry and Endodontics, at Anil Neerukonda Institute Of Dental Sciences, Visakhapatnam, Andhra Pradesh from August 2023 to November 2023. A total 50 human maxillary premolars were taken in this study.

Study Design: Invitro original research

Study Location: Department of Conservative Dentistry and Endodontics, at Anil Neerukonda Institute Of Dental Sciences, Visakhapatnam, Andhra Pradesh

Study Duration: August 2023 to November 2023.

Sample size: 50 human maxillary premolars.

Subjects & selection method: 50 human maxillary premolars removed for orthodontic or periodontal reasons, were collected from Anil Neerukonda Institute Of Dental sciences, Visakhapatnam from June 2023 to July 2023.

Inclusion criteria:
1.Human maxillary central incisors were selected.

Exclusion criteria:
1 Cracks And Craze Lines

2 Crown Fractured Tooth
3 Hypoplastic Teeth
4 Internal Resorption
5 Tooth With Dental Fluorosis
6 Radiographic Evidence Of Endodontically Treated Teeth

Procedure methodology

This study was conducted in August 2023 and the duration was around 2 months. All the samples were cleaned with an ultrasonic scaler to remove the surface deposits and polished with pumice to remove plaque and debris. Later they were immersed in a 0.1% thymol solution for disinfection and storage until the experimental period. A 4 × 4 mm label placed on buccal surface of the crown which was measured using vernier callipers. Remaining portion of tooth was painted with nail varnish. The samples were subjected to a demineralizing solution

Preparation Of Demineralizing Solution:

The demineralising solution is made up of using 0.264 gm of monosodium phosphate,0.244 gm of calcium chloride, 2.86 ml of acetic acid. All these are mixed in 1 litre of deionised water to prepare a demineralizing solution.

Now samples are subjected to demineralizing solution for a period of 4 days to obtain an artificial white spot lesion followed by placement of samples in deionized water for 24 hr. The specimens will be air dried and subjected to energy dispersive x ray spectroscopic analysis for calculation of calcium and phosphorus wt %.

Grouping of specimens:

The results of EDX are obtained after demineralization and tabulated and the same samples are now subjected to remineralization using different remineralizing agents. The specimens are now divided into 5 groups namely:

Group 1: CPP-ACP
Group 2: Egg shell solution
Group 3: Red Marine algae
Group 4: Bioactive Glass Nanoparticles
Group 5: Acclaim tooth paste

FIG 1: Collection Of Samples And Demineralizing And Remineralizing Agents

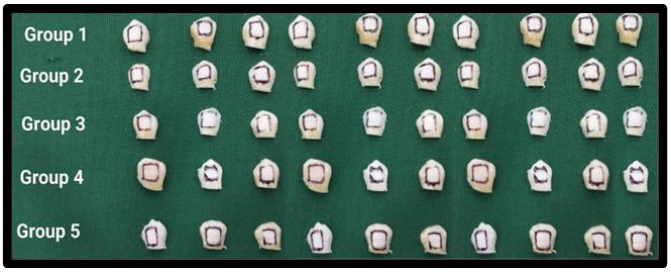


FIG 2: Grouping Of Samples

Method of application of remineralizing agents:
Group 1: CPP-ACP
10% CPP-ACP paste is applied with brush for 5 minutes and rinsed with water twice a day.

Group 2: Egg shell solution
One gram of egg shell powder is mixed with 20 ml of 4% acetic acid and supernatant was collected and the samples were immersed in this solution.

Group 3: Red Marine algae
20 ml of 4% acetic acid mixed with 1 gm of algae powder, supernatant collected and the samples were immersed in this solution

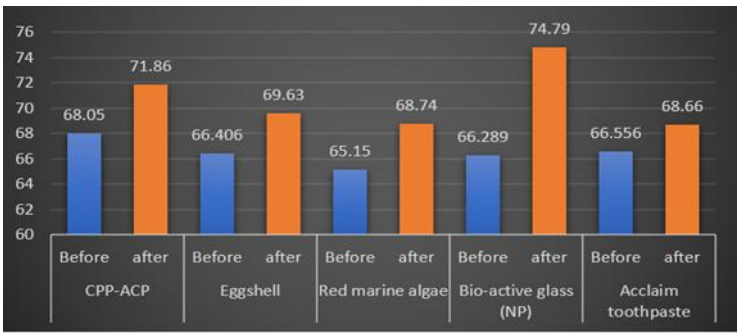
Group 4: Bioactive Glass Nanoparticles
150 mg of bioactive glass nanoparticles will be suspended in 0.87 ml of water. The specimens will be immersed into this solution.

Group 5: Acclaim tooth paste
Acclaim toothpaste applied on samples using applicator tip and immersed in water.
After application of respective remineralizing agents to the specimens for 7 days. After 7 days the specimens are cleansed with distilled water and will be air dried. Now the samples are subjected to energy dispersive x ray spectroscopic analysis. The surface characteristics of remineralized enamel will be evaluated and the ca/p wt% will be calculated and tabulated.

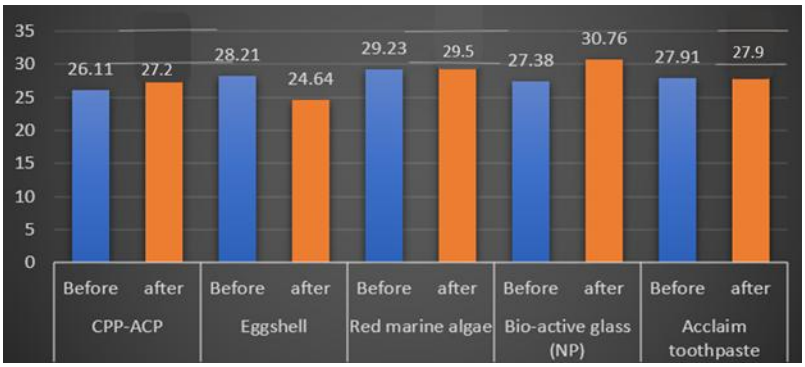
Statistical analysis
The Obtained data which is collected and is analysed using SPSS software. The Data analysed by ANOVA at a significance level of 0.05%. Post hoc tests were conducted to determine the significant difference between groups. Data will be entered in the excel spread sheet. The mean and standard deviations for the ca and p values will be obtained for five different remineralizing agents. A descriptive analysis of the data will be presented as frequency, mean
One- way ANOVA will be used to compare the mean scores of the different parameters and post hoc will be done for comparisons between the subjects. The level of significance will be set at $P<0.05$.And any other relevant test, if found appropriate during the time of data analysis will be dealt accordingly.

III. Results

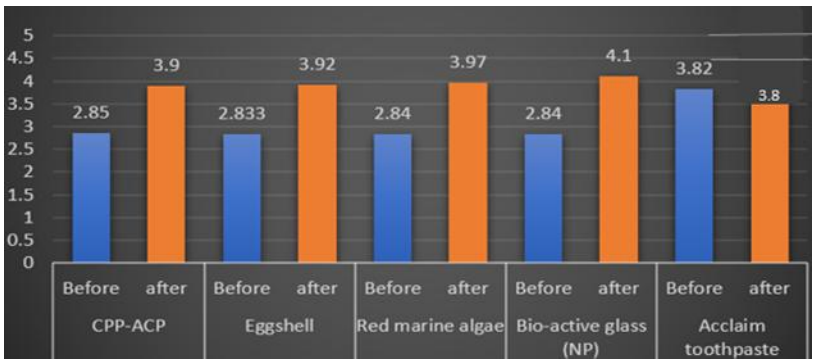
The intergroup comparisons have shown a significant difference in the measured parameters which is the remineralizing ability to the different remineralizing agents. The calcium, phosphate and fluoride values of group 2 (egg shell solution) has shown the least results indicating that it has the least remineralizing ability. The ca, p, f values of group 3 (red marine algae) has shown better results compared to egg shell solution. Group 4 (bioactive glass nanoparticle solution) has shown the best remineralizing ability among all other remineralizing agents by increasing the calcium, phosphate and fluoride values of demineralized teeth. The second highest remineralizing ability is shown by group 5 (acclaim toothpaste). The group 1 (CPP-ACP) has shown intermediate results in increasing the ca, p and fluoride values.
The mean remineralizing ability of group 5 (bioactive glass is the glass) has shown the highest results of all other groups indicating that BAG can be best used for reversal of initial carious lesions.



Graph 1: Graph Represents The Comparison Of Calcium Levels Before And After Remineralisation



Graph 2: Graph Represents The Comparison Of Phosphate Levels Before And After Remineralisation



Graph 3: Graph Represents The Comparison Of Flouride Levels Before And After Remineralisation

IV. Discussion

The study was conducted to compare and evaluate the remineralizing ability of five different remineralizing agents namely CPP- ACP, egg shell powder, red marine algae, bioactive glass nanoparticles and acclaim toothpaste. The purpose pd the stidy was to undersatand the reversal eddects of demineralized enamel caused due to acid attack caused by bacteria. The study was conducted to aquire comprehensive knowledge and insights about the charecteristics, functions and applications of various remineralizing agents. By comparing and analyzing the remineralizing ability of various agents, this study aims to contribute to thye field of minimally invasive dentistry, and enhances the understanding of patient needs and more successful treatment outcomes.

There are several factors to be considered when selecting materials for a study, such as the relevance to the research, availability, clinical applicability, and previous research evidence. A thorough literature review is done to identify suitable materials that have been previously used in similar studies or have demonstrated favorable outcomes. The choice of materials should align with the study's objectives and aim to address any limitations in the literature.

Decreased ph of oral cavity to a level below that of critical ph leads to removal of ca, p and f thereby leading to demineralization and appearance of a which spot lesion which may further progress to dental caries. Therefore various newer remineralizing agents have been invented in the last few decades.

The first group chosen for study is Casein phosphopeptides-amorphous calcium phosphate (CPP-ACP) is a bioactive agent with a base of milk products, which has been formulated from two parts: casein phosphopeptides (CPP) and amorphous calcium phosphate (ACP). CPP was produced from milk protein casein and has a remarkable ability to stabilize calcium phosphate in solution and to substantially increase the level of calcium phosphate in dental plaque. CPP-ACP buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to tooth enamel, reducing demineralisation and promoting remineralisation. The free calcium and phosphate ions move out of the CPP, enter the enamel rods and reform onto apatite crystals. CPP-ACP is useful in the treatment of white spot lesions, hypomineralised enamel, mild fluorosis, tooth sensitivity and erosion, and prevents plaque accumulation around brackets and other orthodontic appliances. The disadvantage with cpp acp includes the lower hardness value. This is due to the amorphous nature; which does not adhere to the enamel surface, hence not remineralizing the tooth surface for a longer period of time to enhance its hardness.

The bioactive glass nanoparticles and is effective against bacteria and prevents progression of white spot lesion. BAGnp leads to deposition of crystal-like hydroxyapatite (HAP) rich in calcium and phosphate with the presence of silica. This layer of mineral formed by the particles of bioactive glass not only covered the lesion surface, but also partially or completely occluded dentine tubules during remineralization The physical occlusion

on the lesion surface begins with the bioactive glass particles exposed to the aqueous environment, along with ion release and pH elevation . When the biomaterial is exposed to an aqueous environment, sodium ions will exchange with H^+ (hydrogen ions). Meanwhile, Ca^{2+} (calcium ions) in the particles as well as PO_4^{3-} (phosphate ions) are released from the biomaterial. Thus, a localized pH rise will allow the precipitates of calcium and phosphate ions, together with the ions from saliva to form a calcium phosphate (Ca–P) layer on the lesion surface.

The silica network from bioactive glass can react with hydroxyl ions from aqueous solution and form soluble silanol compounds. It can be observed that the increase in Ca and P content would induce a decrease in Si content. The newly formed layer displays good resistance to abrasion and transforms to a hydroxyapatite layer ultimately, which is structurally similar to those of original enamel and dentin.

Feroz et al stated that ESP (egg shell powder) application reduced the enamel surface roughness. A state of supersaturation is maintained because of the bioavailability of the calcium and phosphate ions, which could enhances the remineralization process of the enamel surface due to rapid precipitation of the minerals on the enamel surface filling all the surface micro porosities ending with their closure and hence enamel remineralization. ESP has increased microhardness and decreased enamel surface roughness and it could be used effectively as a remineralizing agent. As it promotes remineralization and prevents demineralization, it also acts as a reservoir of ions, so it might be efficient to remineralize the demineralized enamel surface.

LC to performs similar to fluoride in supporting enamel hardness recovery and mineral gain. The remaining skeleton of seaweed LC is a natural source of calcium (Ca), magnesium (Mg) and several other mineral elements. Even if Mg concentrations in mature enamel are low, and reported to range from 0.4% in the inner enamel layer to 0.1% near the outer enamel surface, its presence in the fluid surrounding enamel is key for enamel development and mineralization. Accordingly, Mg is expected to play an adjuvant but significant role in therapeutical schemes aiming at either the remineralization of mature demineralized enamel, or prevention of intact enamel demineralization. The multi-oligoelemental composition of LC may further act in synergism with Ca and Mg to assist enamel remineralization.

The last group chosen is acclaim toothpaste which contains nanohydroxyapatite. Nano-hydroxyapatite can provide a source of calcium for the oral cavity; increased calcium levels can help to limit the acid challenge, reducing enamel demineralization while promoting enamel remineralization. This calcium phosphate reservoir may contribute to an enamel mineral oversaturation state, hence decreasing demineralization and enhancing remineralization. It has also been found that nHAp decreased caries susceptibility, enhanced enamel remineralization, inhibited caries, and reduced dentin demineralization. Possibly, this could be due to the deposition of a new homogenous apatite surface layer on the demineralized surface. This mechanism protects the underlying diseased surface from further demineralization and promotes remineralization. Nano-hydroxyapatite also promotes more minerals to be accumulated in the outer layer of carious lesions, thus resulting in a highly mineralized external layer and inhibiting mineral ions from entering deeper regions of the demineralized lesion.

V. Conclusion

Based on the results it was concluded that the bioactive glass nanoparticle have shown better remineralization efficacy by increasing the amount of calcium, phosphate and fluoride. BAGnp is followed by acclaim toothpaste then is the CPP- ACP and the least amount of ca, p and f valued are shown by red marine algae and egg shell solution.

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