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Effects of Remineralising Agents on Attrited Teeth- An in Vitro Study

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Abstract: ***The study is about evaluating the effects of three remineralising agents i.e. Casein Phosphopeptide - Amorphous Calcium Phosphate [CPP-ACP], Sodium fluoride varnish and Tricalcium phosphate (TCP) on dentin. The objective of this study is to see which remineralising agents have a better effect on dentin. In this study, thirty freshly extracted human central incisors are selected with exposed dentin. The labial surfaces of each tooth are divided into four quadrants and treated with the following agents. They are as follows:- Part A is used as a Control unit, Part B is applied with Tricalcium phosphate (TCP), Part C is applied with Casein Phosphopeptide - Amorphous Calcium Phosphate [CPP-ACP] and Part D is applied with Sodium fluoride varnish. Then the teeth will be examined under the microscope and evaluated accordingly. The purpose of this study is to compare the effects of three remineralising agents i.e.Casein Phosphopeptide - Amorphous Calcium Phosphate [CPP-ACP], Sodium fluoride varnish and Tricalcium phosphate (TCP) on dentin and layering a new coat of tooth surface on attrited surface.***

Keywords: Casein Phosphopeptide - Amorphous Calcium Phosphate, Sodium fluoride varnish, Remineralisation, Tricalcium phosphate.

I. INTRODUCTION

Remineralisation is defined as the process whereby calcium and phosphate ions are supplied from a source external to the tooth to promote ion deposition into crystal voids in demineralised enamel to produce net mineral gain.[1]Remineralisation of teeth is a naturally occurring process in the oral cavity and does not have only one reason – tooth decay. There are bacterial as well as non-bacterial causes responsible for dentin exposure; attrition being the most common cause. Attrition is said to be a physiologic process as it is a natural outcome of ageing but still, its rate and severity depend on diet quality, dentition, and force of the masticatory muscles. As attrition can occur in both deciduous and permanent dentition, it is clinically manifested by the formation of flat, shiny, smooth, well-polished surfaces of teeth. Remineralising agents are adjunct to preventive dentistry. They help in remineralising enamel and dentin. The process of remineralisation is governed by the saturation of oral fluids.

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There are different modes of delivery of remineralising agents those are dentifrices, mouth rinses, lozenges or chewing gums.[2]Thus, the present study aims to compare and evaluate the effects of remineralising agents on artificially attrited teeth.

II. MATERIALS AND METHOD

Materials used: - Artificial saliva, Applicator tip, Etching gel, SEM, Ultrasonic scalerand Remineralising agents.The remineralising agents used are Casein Phosphopeptide - Amorphous Calcium Phosphate [CPP-ACP], Sodium fluoride varnish and Tricalcium phosphate (TCP).

III. METHOD

A. Specimen selection and preparation

30 attrited incisors were selected as specimens. The teeth were non-carious but attrited and whose dentin was exposed. All soft debris and calculus undergo thorough scaling with an ultrasonic scaler.[3]

B. Preparation of specimens for demineralisation

Specimens are then artificially demineralised for 48 hours in acetic acid. The pH of acetic acid was approximately below 5.5pH. Acid etching was also done for 10-15 seconds. Then the labial aspect of the crown of specimens was sectioned into four quadrants by high-speed straight bur.

In a clockwise direction, the 4 quadrants were divided into:-
Quadrant 1 was made as a Control unit.

Quadrant 2 was remineralised by Tricalcium Phosphate (TCP).(CLINPRO Tooth crème, 3M).

Quadrant 3 was remineralised by Casein Phosphopeptide-Amorphous Calcium Phosphate [CPP-ACP]. (GC Tooth Mousse)

Quadrant 4 was remineralised by Sodium Fluoride Varnish.(Prime).

Tricalcium Phosphate

Tricalcium phosphate (TCP) is a new hybrid material created with a milling technique that fuses beta-tricalcium phosphate (ί-TCP) and sodium lauryl sulfate or fumaric acid. This blending results in a "functionalized" calcium and a "free" phosphate, designed to increase the efficacy of fluoride remineralisation.[4]Tricalcium phosphate (TCP) is a remineralising agent which plays a role in increasing the free calcium concentration in saliva and dental plaque.[5]TCP breaks the protective barrier, is moistened by saliva as it comes in contact with the tooth and thus facilitates the availability of calcium and phosphate.[6]

Casein Phosphopeptide-Amorphous Calcium Phosphate [CPP-ACP]

Casein Phosphopeptide-Amorphous Calcium Phosphate [CPP-ACP] was introduced as a remineralising agent in the year 1998 and claims to promote remineralisation by maintaining a supersaturated state of essential minerals.[3]Casein Phosphopeptide-Amorphous Calcium Phosphate [CPP-ACP] is believed to have an antibacterial and buffering effect on plaque.[6]Casein, a milk phosphor protein, interacts with calcium and phosphate. As the pH of the material increases, the bound form of amorphous calcium phosphate increases thereby stabilizing free calcium and phosphate.[7]

Sodium Fluoride VarnishFluoride is recognised as a remineralising agent, which interacts with the oral fluids on the interface of enamel and subsurface region of teeth and combines with calcium and phosphate ions to form fluorapatite.[3]Sodium fluoride varnish can be applied topically two to four times a year.

Artificial Saliva

Saliva plays an important role in chewing, swallowing, digestion and speaking. It also helps control bacteria in your mouth, which helps prevent infection and tooth decay.The use of artificial saliva was reported for the first time by W. Souder and W.T. Sweeney in the year 1931.[8] Freshly made artificial saliva was used to create an oral cavity environment and promote remineralisation. The artificial saliva used had a 6.7 pH.

C. Treatment on specimens

The remineralising agents were applied by applicator tip to the quadrants: - Tricalcium phosphate was applied to quadrant 2, Casein Phosphopeptide-Amorphous Calcium Phosphate was applied to quadrant 3 and Sodium fluoride varnish was applied to quadrant 4. The first quadrant was left untouched as it was the control unit. Once it is done the teeth were kept in artificial saliva. Artificial saliva was changed every day to enhance its effectivenessas its pH used to differ over the due course of time. This regimen was continued for 30 days at room temperature.The artificial saliva had a mineral content devoid of calcium (i.e. xylitol, potassium chloride, sodium chloride, magnesium, potassium phosphate and sodium saccharin) that was changed daily.[9]

D. Statistical Analysis

After completing the cycle of 30 days the teeth were assessed under a scanning electron microscope [SEM] to estimate the mineral content (calcium, phosphate and fluoride). Every quadrant was checked under 500x, 1000x, 2000x and 5000x SEM-EDAX.

IV. RESULT

After the following cycle of 30 days and SEM-EDAX evaluation specimens of different groups showvast alterations in enamel.

SEM image of quadrant 1- Control unit

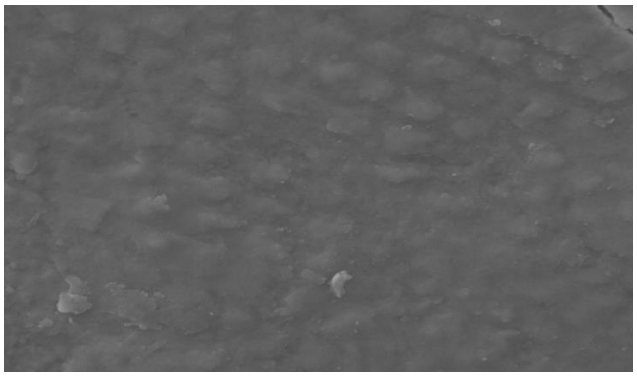
SEM image of quadrant 2-Tricalcium phosphate

SEM image of quadrant 3- Casein Phosphopeptide-Amorphous Calcium Phosphate

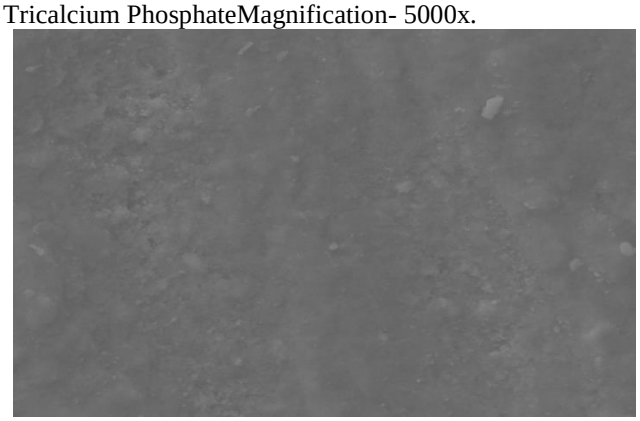
SEM image of quadrant 4- Sodium fluoride varnish

1.) Post-demineralization/pre-remineralisationkeyhole appearance is seen.

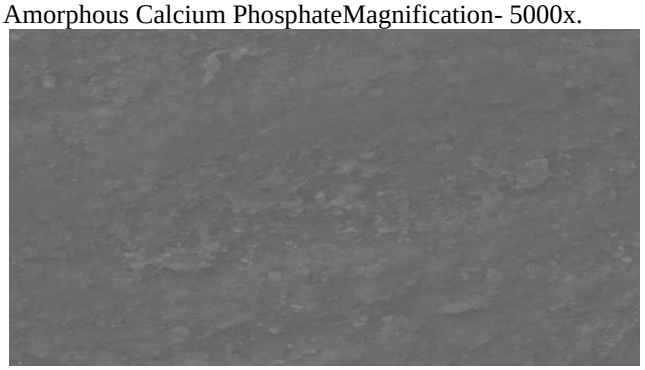
SEM image of quadrant 1- control unit Magnification- 5000x.



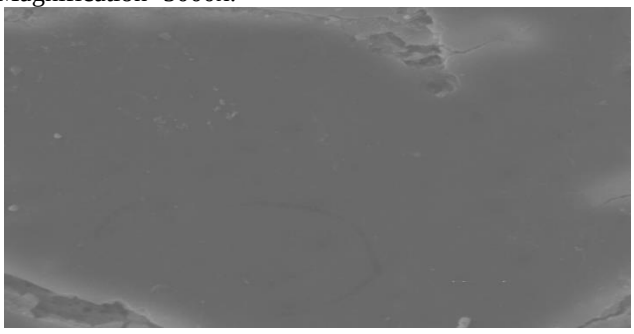
2.)Post remineralisation SEM image of quadrant 2- Tricalcium PhosphateMagnification- 5000x.



SEM image of quadrant 3- Casein Phosphopeptide-Amorphous Calcium PhosphateMagnification- 5000x.



SEM image of quadrant 4- Sodium fluoride varnish Magnification- 5000x.



On observation, there is a change in the surface of the enamel.The initial enamel shows micro pores under 5000x magnification.

The latter enamel surfaces remineralised by Tricalcium phosphate, Casein Phosphopeptide-Amorphous Calcium Phosphate and Sodium fluoride varnish show re-established surface integrity and covered micro pores and cavities. The result also shows varied densities of enamel remineralised by different agents.

For the SEM examination, four sample specimens in each group were randomly selected and evaluated for surface changes. The scanning electron microscope was used to determine and compare the morphological variations between the differently treated samples. For comparison, the surfaces of the sound and demineralised enamel were also examined. Images were obtained at 500x, 1000x, 2000x and 5000x magnifications under a microscope.

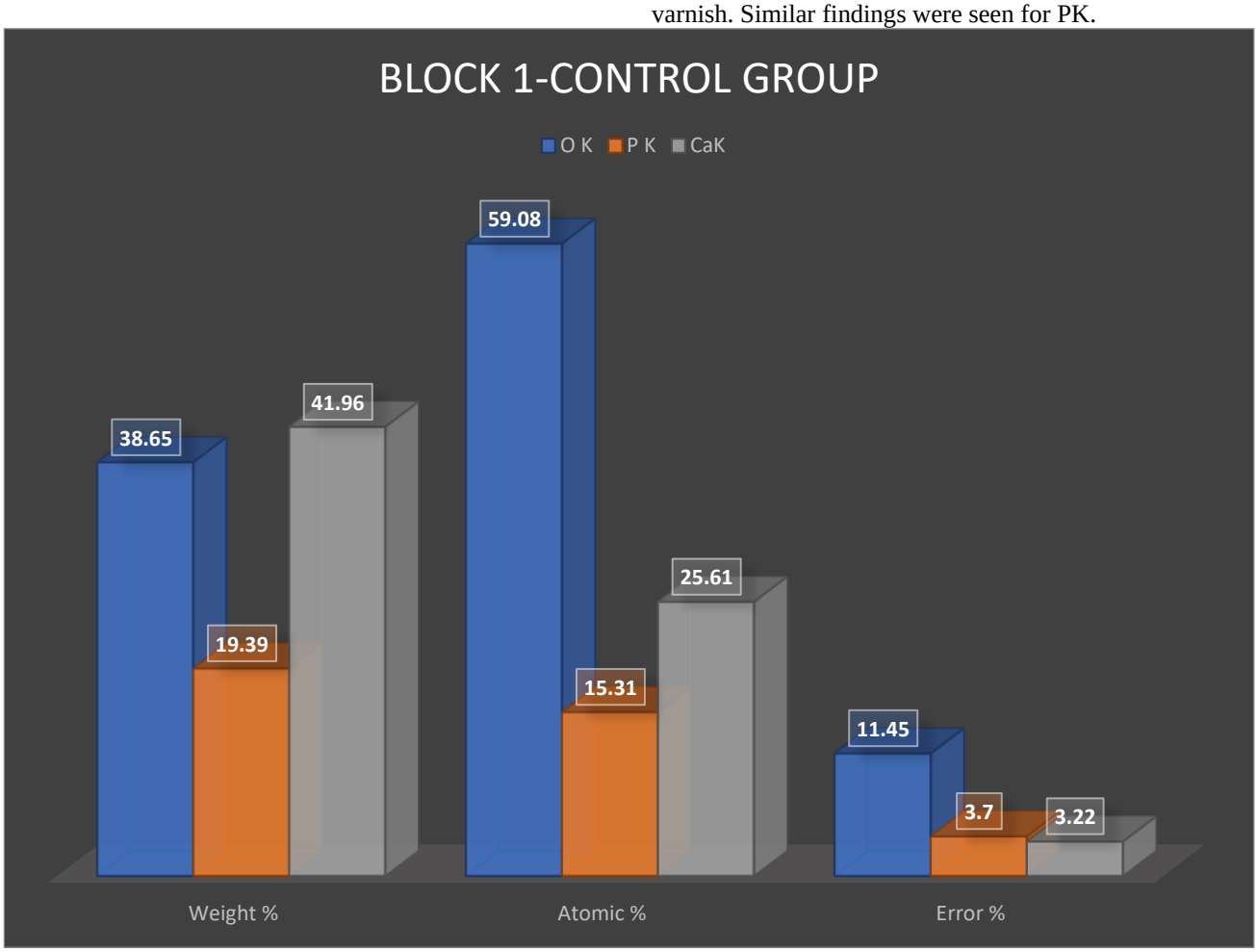
Block 1 was quadrant 1 of the control group had OK, CaK and PK analysed for their atomic% and weight%. OK was 38.65% and 59.08%, CaK was 41.96% and 25.61% while PK was 19.39% and 15.31% as their atomic and weight % respectively. The total weight % and atomic % were 61.35 and 40.92 with a cumulative error% of 6.92.

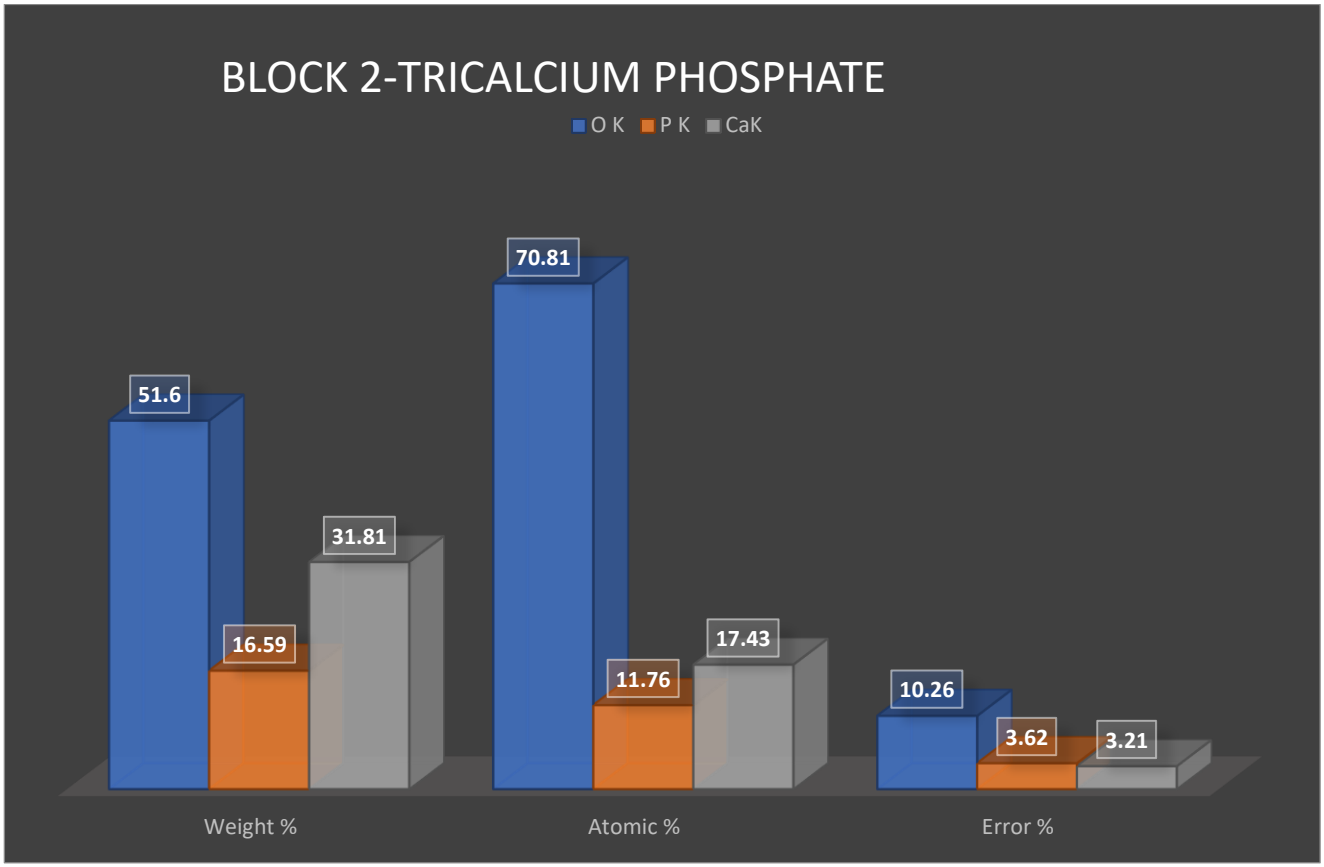
Block 2 assessed CaK and PK of quadrant 2 treated with Tricalcium phosphate indicating a weight% of 31.81 and atomic% of 17.43 for CaK whereas PK was 16.59% and 11.76%. The OK values were 51.60% and 70.81% for weight and atomic %. The mean weight% was 48.4 and 29.19 as atomic% with a mean error % of 6.83.

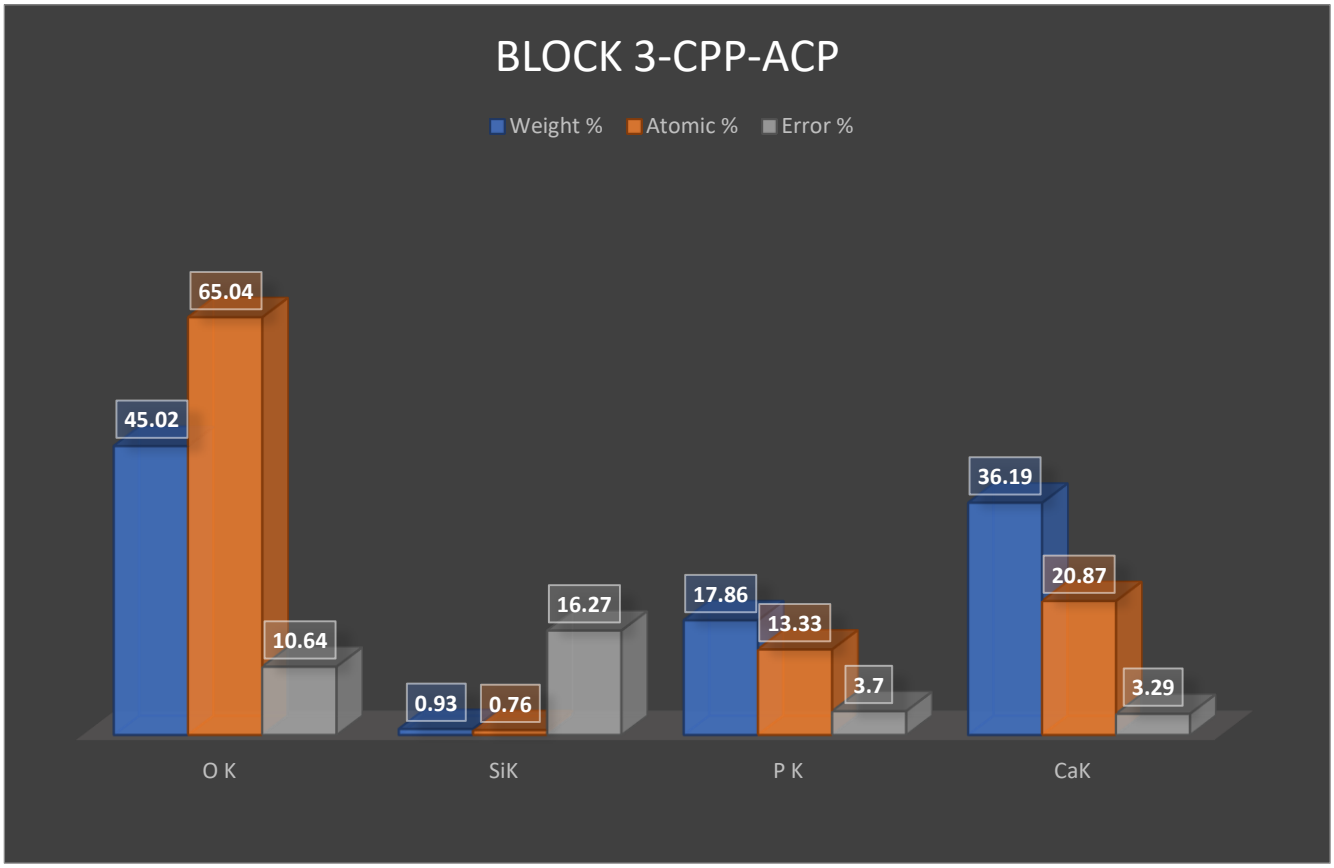
Block 3 assessed similar findings for CPP-ACP. The CaK was 36.19 and 20.87 at weight% and atomic% while the PK was 17.86 and 13.33 at weight% and atomic%. SiK was also assessed for this group, the weight and atomic % were 0.93 and 0.76%. The cumulative weight% and atomic% were 54.05 and 34.2, the error% was 6.99.

Block 4 assessed NaK along with CaK and PK for the effect of sodium fluoride varnish. The weight% and atomic% were 40.33 and 23.07-CaK, 0.18 and 0.18-NaK and 18.87 and 13.97-PK. The weight and atomic% for SiK were 1.01% and 0.47%. The total weight % and atomic % were 59.38 and 37.22 with a cumulative error% of 79.

The CaK levels were highest in the control group by weight and atomic levels while it was lowest for sodium fluoride varnish. Similar findings were seen for PK.







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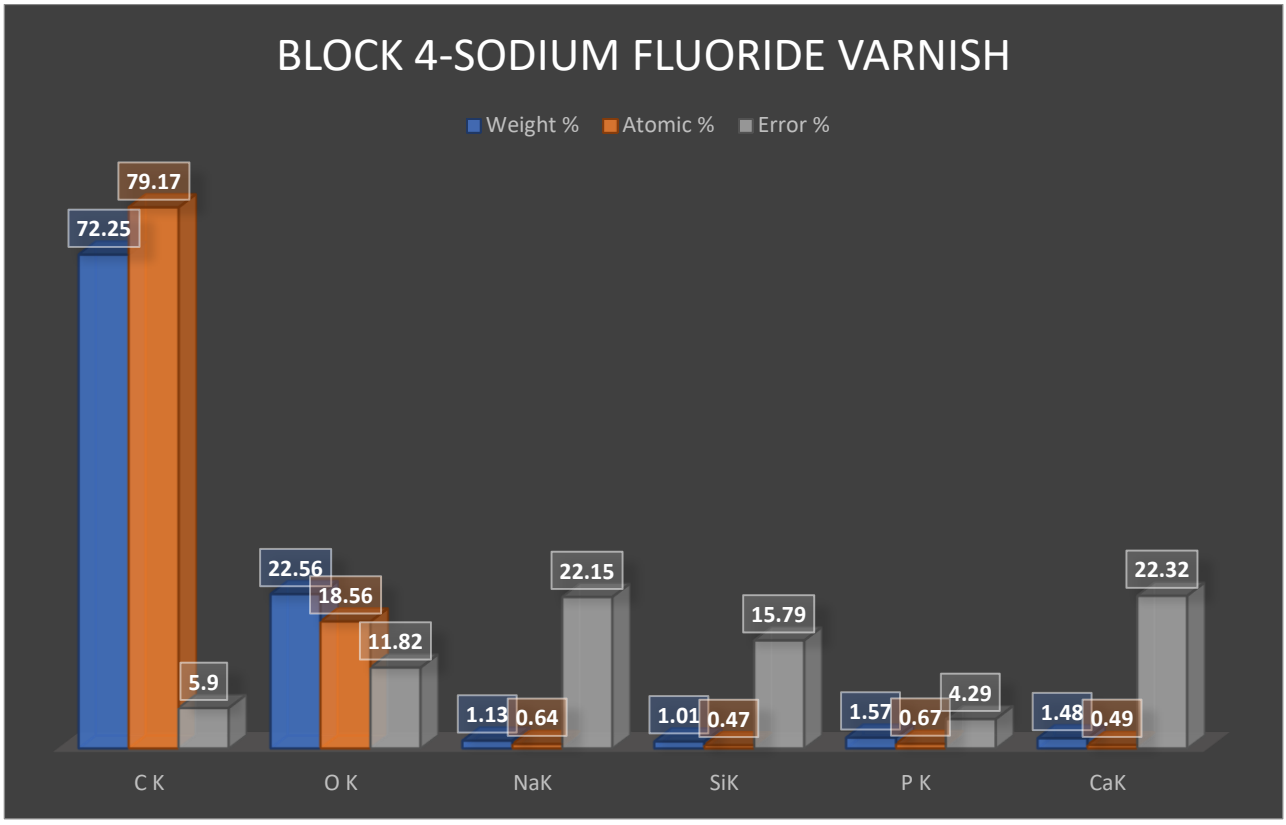

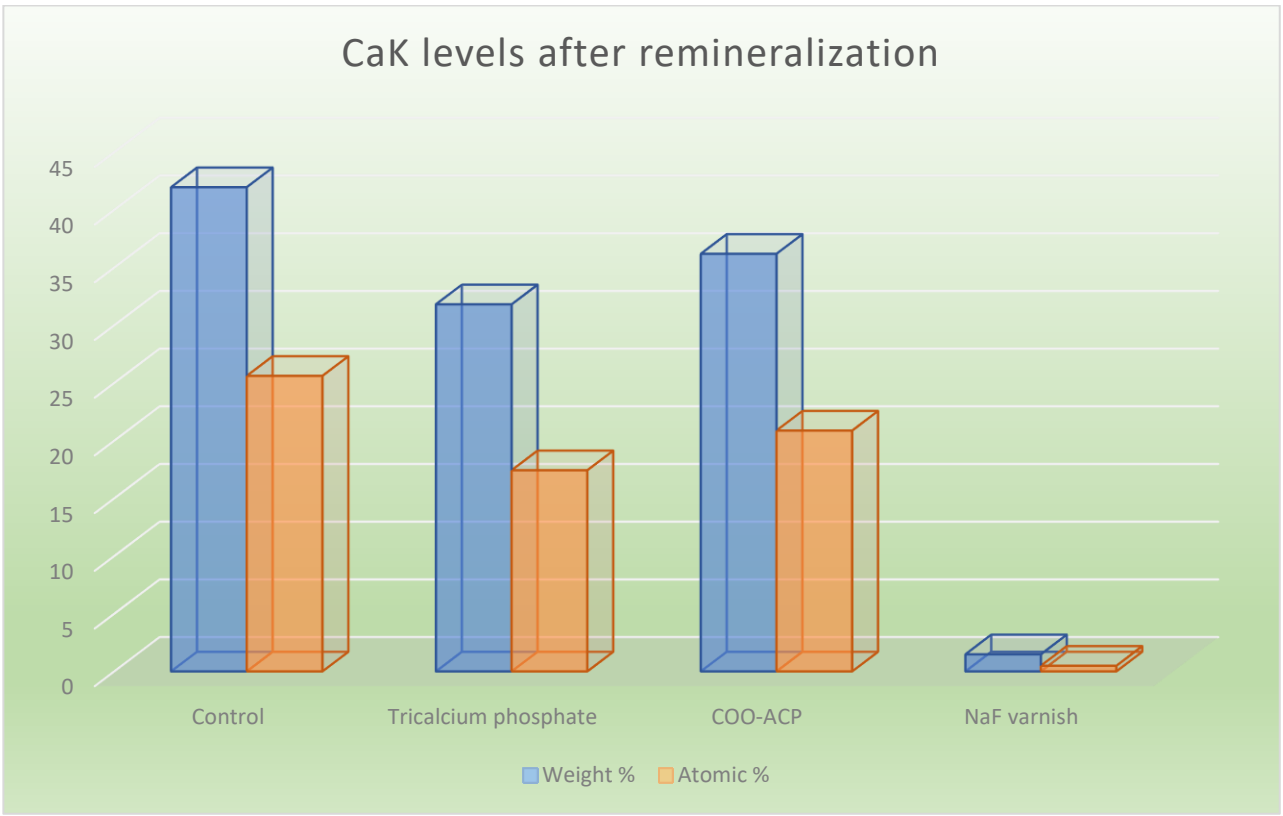

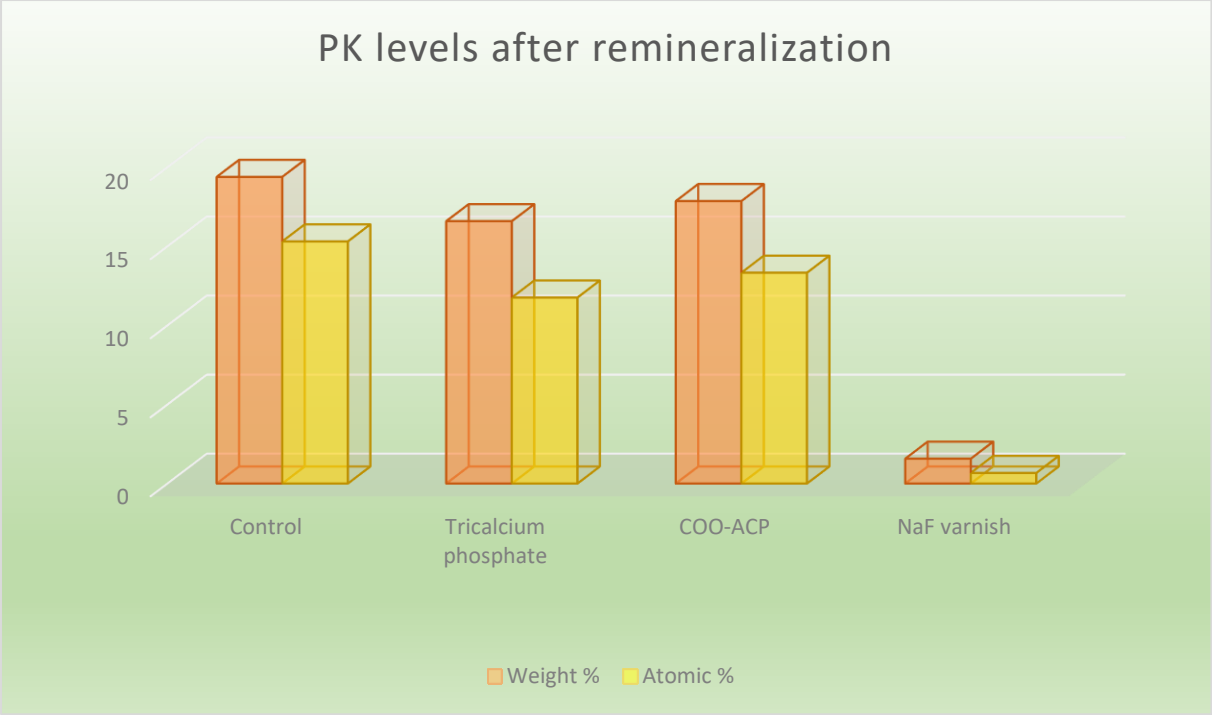

V. DISCUSSION

Attrition is mostly caused by tooth to tooth contact. Bruxism, clenching, developmental defects, coarse diet, etc are some reasons for the attrition of teeth. Attrition is mostly seen on the incisal and occlusal surfaces of the teeth. There is a loss of tooth structure and amplified dentin hypersensitivity. Tooth discolouration, tooth mobility due to compromised periodontal support, malocclusion as vertical height decreases, and failure of the restoration.

The present in vitro study showed that three different remineralising agents vary in their capability to remineralise the hard tissue of the tooth. It also prevented calculus formation and increased remineralising property of saliva.

Sodium fluoride is the most effective and has the highest capability to deposit calcium and phosphate. It also prevents calculus formation and increases the remineralising property of saliva. Fluoride has been studied for decades and has shown to be effective in preventing by inhibiting demineralization, enhancing remineralisation, and reducing the metabolic activity of bacteria.[10]There are many studies in which the remineralisation efficiency of sodium fluoride varnishes was evaluated in vitro which showed sodium fluoride varnish shows a remarkable increase in enamel remineralisation.[11]Tyagi et al. suggested Tricalcium phosphate (TCP) is similar to apatite structure and possesses unique calcium environments capable of reacting with fluoride and enamel. While the phosphate floats free, these exposed calcium environments are protected, preventing the calcium from prematurely interacting with fluoride. Tricalcium phosphate (TCP) provides catalytic amounts of calcium to boost fluoride efficacy and may be well designed to coexist with fluoride in a mouth rinse or dentifrice because it will not react before reaching the tooth surface. When TCP finally comes into contact with the tooth surface and is moistened by saliva, the protective barrier breaks down, making the calcium, phosphate and fluoride ions available to the teeth. The fluoride and calcium then react with weakened enamel to provide a seed for enhanced mineral growth relative to fluoride alone.[4]The calcium and phosphate come primarily from saliva but it does not exert a uniform effect in the mouth and they mostly bring about distinct localized effects. So to keep away from such side effects fluoride treatment has been strongly advised.[9] As it was an in vitro study artificial saliva was used to mimic oral environment calcium and phosphate concentrations and the corresponding calcium-to-phosphate ratios, in the artificial saliva,may be higher than in physiological saliva.[12] Schemehorn et al. has also suggested that the addition of ACP rather than tricalcium phosphate may provide an even greater increase in the ability of fluoride varnish to prevent caries. The anti-cariogenic mechanism of CPP-ACP may involve the incorporation of nanocomplexes into plaque and onto the tooth surface. These localized CPP-ACP nanocomplexes have been purported to buffer the activity of free calcium and phosphate ions, thereby maintaining a state of supersaturation with respect to tooth enamel, preventing enamel demineralization and promoting remineralisation. Pithon et al. reported that MI varnish (5% sodium fluoride varnish with added CPP-ACP) was more effective than Duraphat (5% sodium fluoride varnish without CPP-ACP) in reducing the depth of caries lesions around orthodontic brackets. Moreover, a recent study by Schemehorn et al. found varnish containing ACP to promote significantly higher levels of fluoride deposition into enamel when compared to a varnish containing tricalcium phosphate.

Cochrane et al. in a study evaluating the release of fluoride, calcium and inorganic phosphate ions from fluoride varnish with different material compositions (MI, Clinpro White, Enamel Pro, Bifluorid 5, Duraphat), found calcium and fluoride ion release to be highest with MI; furthermore, no inorganic phosphate ion release was observed with either Bifluorid 5 or Duraphat. Previous SEM studies have reported a homogeneous remineralized layer on enamel surfaces treated with CPP-ACP paste.[13]Topical fluoride varnishes are well-known and frequently used remineralising agents in enamel caries lesions. There are many studies where remineralising efficiency of fluoride varnish was evaluated in vitro and in situ which showed that sodium fluoride varnishes remarkably increased enamel remineralisation.[14]The proposed anti-cariogenic mechanism for CPP–ACP is by the localization of ACP on the tooth surface, which buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to the tooth enamel and thus preventing demineralisation and enhancing remineralisation.

VI. LIMITATIONS

In this in vitro study, the precise and dynamic biologic systems and conditions ofthe oral cavity in vivo needed for remineralisation can not be met in full magnitude. To name a few the roles of enzymes are not taken into account and the effects of salivary proteins, pellicle and plaque on remineralisation inhibition are also not taken into consideration. To add to my in vitro study limitation there isa possibility of experimental errors and dissimilarities in the micro-structure of the enamel between specimens. Nevertheless, a straightforward assumption must be made with caution due to the constraints of in vitro studies.

VII. CONCLUSION

After considering the results and limitations of this present study it shows that all remineralising agents were effective. A layer of remineralised enamel is formed and can be seen under a scanning electron microscope [SEM]. Sodium fluoride varnish deposits the highest amount of calcium and phosphate on attrited subsurface. It gives a much better effect than Tricalcium phosphate (TCP) and Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP).Application to the general population requires further research and analysis.

DECLARATION

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Ethical Approval and Consent to Participate	No, the article does not require ethical approval and consent to participate with evidence.
Availability of Data and Material/ Data Access Statement	Not relevant.
Authors Contributions	All authors have equal participation in this article.

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My publications:- Effect of Preoperative Oral Steroids in Comparison to Anti-inflammatory on Anesthetic Success of Inferior Alveolar Nerve Block in Mandibular Molars with Symptomatic Irreversible Pulpitis- A Double-blinded Randomized Clinical Trial and Prevalence of dens invaginatus and its association with periapical lesions in a Western Indian population- a study using cone-beam computed tomography.