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Enamel Armour: Mineral Magic Behind Restoring Tooth Structure

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ABSTRACT

Aim

To evaluate and compare the surface microhardness of artificially demineralized teeth treated with Nanohydroxyapatite and Casein Phosphopeptide - Amorphous Calcium Phosphate remineralising agents.

Materials and methods

Twenty intact caries free maxillary premolars were taken for the study. The tooth were decoronated at the level of CEJ and further divided into 2 portions. The samples were polished and divided into Group A(control), B(demineralized), C(remineralised with nanohydroxyapatite) and D(remineralised with CPP-ACP). All test group samples were demineralized by immersing in demineralizing solutions for 96 hours. Afterwards, group C and D samples were

remineralized by remineralizing agents (Aclaim and GC Tooth Mousse) for three minutes (twice a day) for 14 days, and then Vickers microhardness testing (VHN) was performed.

Results

The microhardness values of the demineralized group were lower compared to the samples of the control groups. In the remineralized group, the mean microhardness values were maximum for Aclaim (Nanohydroxyapatite) [368 ± 9.66], followed by GC Tooth Mousse (CPP-ACP) [319.4 ± 15.8].

Conclusion

The application of remineralizing paste proved potent in improving the remineralization of the demineralized enamel surface.

Keywords

Dental caries, Demineralization, Microhardness, Remineralisation.

INTRODUCTION

Enamel forms the outermost surface of the tooth, which is highly mineralised and serves as the primary protective barrier against mechanical forces, temperature changes and chemical erosion. It is the hardest and most resilient tissue in the human body, consisting of approximately 96% inorganic material (primarily hydroxyapatite crystals), 3% water, and 1% organic matrix by weight [1,2]. The high mineral content contributes to its remarkable hardness and brittleness, while the limited organic content restricts its capacity for self-repair once damaged.

Structurally, enamel is composed of tightly packed enamel prisms / rods and interprismatic substance, arranged in a highly ordered pattern that enhances its mechanical strength and resistance to fracture [3]. Despite its durability, enamel remains susceptible to demineralization caused by acids produced from bacterial metabolism or dietary sources, leading to dental caries [4]. Because of this, enamel integrity is critically dependent on the balance between mineral loss (demineralization) and mineral gain (remineralization) in the oral environment [5].

Enamel demineralization occurs when the pH at the enamel surface drops due to acids produced by bacterial metabolism of fermentable carbohydrates or direct acid erosion [6]. In a healthy oral environment, saliva acts as a buffering medium and contains calcium and phosphate ions, maintaining a dynamic steady state of mineral dissolution and redeposition [7]. When the acid challenge overwhelms the buffering and remineralising capacity, hydroxyapatite crystals begin to dissolve, creating subsurface porosity and clinically the appearance of white-spot lesions (WSLs) [8].

Remineralisation is the natural or augmented process by which mineral ions are redeposited into the demineralised enamel matrix, ideally rebuilding the hydroxyapatite crystals (or more acid-resistant variants such as fluorapatite) and restoring enamel hardness and integrity [9]. The mechanism involves ion diffusion into the micro porosities created by demineralisation, nucleation and growth of new apatite crystals, and crystallite thickening or repair of the compromised lattice [10]. Fluoride plays a pivotal role: when F^- ions are present, they can substitute for the OH^- ions in the hydroxyapatite lattice, forming fluorapatite [$Ca_{10}(PO_4)_6F_2$] or a fluoride-enriched apatite [11], which is less soluble under acid challenge.

Contemporary research has extended remineralisation beyond fluoride alone to biomimetic strategies (e.g., casein-phosphopeptide-amorphous calcium phosphate

[CPP-ACP], bioactive glass, nanohydroxyapatite) aiming for deeper lesion penetration, greater mineral density recovery and more complete lesion repair [12,13].

Various techniques are employed for the assessment of enamel demineralization or remineralisation such as microradiography, wet chemical analysis, light scattering and iodine absorptiometry. In this study, Vicker's Microhardness Number (VHN) testing was used to detect the microhardness in the tooth surface using pyramid shaped indent to obtain accurate measurement both digitally and visually [14].

Hence, this in vitro study aims to assess the remineralising efficacy of Casein - Phosphopeptide - Amorphous Calcium Phosphate [CPP – ACP] and Nanohydroxyapatite by measuring the microhardness of enamel.

MATERIALS AND METHODS

Preparation of samples

A total of 20 extracted maxillary premolars for orthodontic purposes were considered into the study from individuals aged 20 – 30 years. Samples were immersed in a solution of 0.1% thymol for 15 days. The teeth collected were sound teeth, free from any caries, cracks and restorations.

Infection control Protocol

Occupational Safety and Health Administration (OSHA) and Centers for Disease Control and Prevention (CDC) regulations and guidelines were employed in this study to collect, store, sterilize and handle the removed teeth. Standardized infection protocol was followed for the collection of extracted teeth for the study. Calculus was removed from the collected teeth and then sterilized by immersion in 10% formalin for five days followed by 5.25% sodium hypochlorite for five days.

Decoronation and Division of Crown

All sample teeth were decoronated at the level of CEJ and the crown portions were divided into two segments – one buccal and one palatal half using a diamond disc mounted on a straight hand piece.

Fig. 1: Decoronation of sample teeth at the level of CEJ

Fig. 2: Division of crown into two segments

Polishing of Specimens

The exposed enamel surfaces were smoothened with silicon carbide abrasive sheets ranging from 220 – 1000 grit and polished with fine polishing cup and polishing paste to improve the accuracy of microhardness measurements.

Distribution of Specimens

Forty (n = 40) specimens used for the study were randomly allocated into **1** (one) **control group** and **3** (three) **experimental** groups.

Group A	Control group	n = 10 specimens
Group B	Experimental group (Demineralised group)	n = 10 specimens
Group C	Experimental group (Nanohydroxyapatite group – Aclaim)	n = 10 specimens
Group D	Experimental group (CPP-ACP group – GC Tooth Mousse	n = 10 specimens

Preparation of Demineralising Solution

The demineralising solution was prepared with:

- 2.2 mM Calcium chloride solution
- 2.2 mM Monosodium phosphate solution
- 0.05 M Lactic acid solution

To check the pH of the solution, a digital pH meter was used. The pH meter was calibrated by immersing in a buffer solution with a pH of 7.0. The pH of the demineralising solution was raised to 4.5 by addition of 50% Sodium hydroxide solution and the pH of the solution was confirmed with the digital pH meter.

Preparation of artificial caries like lesions

The Amaechi method was used for creation of artificial caries like lesions. All specimens of Group B, C, and D (experimental group; n=30) were submerged in glass containers containing the

demineralizing solution and incubated at 37 °C for 96 hours to induce artificial caries development, encouraging an active region of demineralization [15]. The solution was replaced regularly to avoid mineral build-up from demineralization and the resulting pH shift. The samples were rinsed with deionised water for 30 seconds after 96 hours of demineralisation, dried with an air syringe for 5 seconds, and stored in clean glass containers until further testing.

Remineralising Agents used

Two types of remineralising agents were used for the study:

i. Nanohydroxyapatite
ii. Casein phosphopeptide – Amorphous calcium phosphate (CPP – ACP)

Product	Manufacturer	Composition
Aclaim	Aclaim,™ Bengaluru, India	1% Nanohydroxyapatite
GC Tooth Mousse	Recaldent, GC Japan	10% CPP - ACP

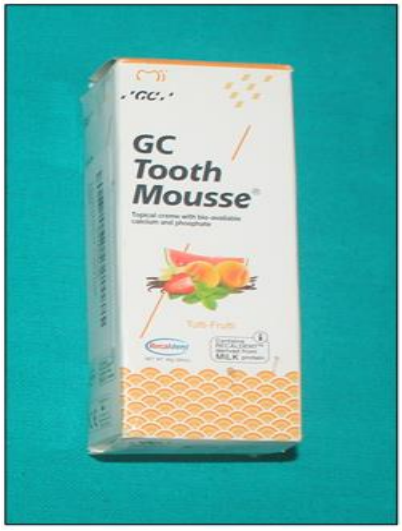


Fig. 3: GC Tooth Mousse (CPP – ACP)

Fig. 4: Aclaim™ (Nanohydroxyapatite)

Nanohydroxyapatite and Casein Phosphopeptide – Amorphous Calcium Pshosphate (CPP – ACP) remineralising agents were applied with soft bristle toothbrush on the exposed demineralised enamel surface of the specific group specimens for three (3) minutes twice a day for 14 days. Each time after application of the remineralising agents, the specimens were rinsed with deionised water for 10 seconds and then stored in artificial saliva (Wet Mouth, ICPA Health Products, India). After 28 cycles of remineralisation for Group C and D, the samples [n = 20]; were taken for microhardness testing using Vicker's Microhardness testing machine.

Vicker's Microhardness Testing

The surface microhardness was measured using VHN testing. The microhardness test was performed, in which pyramid shaped indentations were produced for 20 seconds at a rate of 500-gm load, never close to any edge of the specimen. Three indentations were made on each specimen to assess the specimen's average microhardness. The depth of the indentations were measured, and the values were converted to Vicker's microhardness values.

Fig. 5: Vicker's Microhardness testing Machine

STATISTICAL ANALYSIS

The collected data was tabulated in a spreadsheet using Microsoft Excel 2021 and then statistical analysis was carried out using the GraphPad Prism for Windows, Version 10.1.2 (GraphPad Software, La Jolla California USA). A Shapiro-Wilk's test and a visual inspection of the histograms, standard Q-Q

plots, and box plots showed that the collected data were approximately normally distributed for all the groups. Descriptive statistics were used to report the quantitative variables in terms of mean (central tendency) and Standard deviation (measures of dispersion) along with range. Parametric tests were carried out for inferential statistics. The Analysis of variance (ANOVA) tests were used to analyze differences between groups for various outcome variables, accounting for unequal sample sizes. *Post-hoc* comparisons were conducted using the Tukeys HSD test, with a significance level set at $P \leq 0.05$.

RESULTS

Table 1: Descriptive statistics and Inter-group comparisons of the Microhardness test (in VHN) among the study groups

Study Group	Mean ± SD	Min -Max	*P* value [a]
Group A (Intact teeth, *n*=10)	413.3 ± 21.0 [A]	387.6-442.4	<0.0001*
Group B (Demineralised Teeth, *n*=10)	277.6 ± 17.7 [B]	256.4-301.3	
Group C (Remineralised teeth: NHA, *n*=10)	368 ± 9.66[C]	354.2-382.4	
Group D (Remineralised teeth: CCP-ACP, *n*=10)	319.4 ± 15.8 [D]	294.7-339.3	

n: sample size per group

SD: Standard deviation; Min-Minimum value; Max: maximum value

NHA: Nano Hydroxyapatite, CPP-ACP: Casein phosphuretted–amorphous calcium phosphate

a: analyzed by the Analysis of variance (ANOVA) test

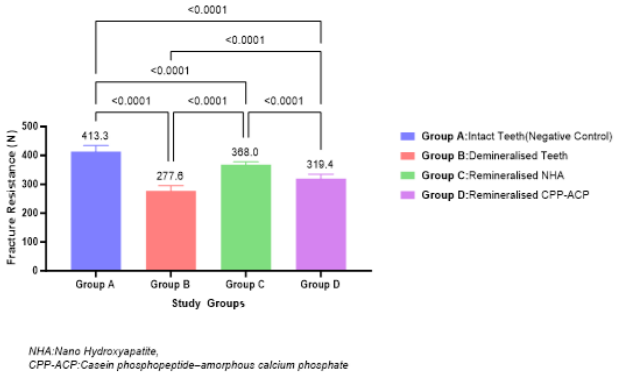
*: statistically significant ($P \leq 0.05$)

Different superscript letters indicate significant differences between the pairs

Table 1 presents the descriptive statistics and inter-group comparisons of microhardness (in VHN) among the study groups. Group A (Intact teeth) demonstrated the highest mean microhardness at 413.3 ± 21.0 VHN, ranging from 387.6 to 442.4 VHN. Group B (Demineralised teeth) exhibited the lowest mean microhardness at 277.6 ± 17.7 VHN, with values ranging from 256.4 to 301.3 VHN. Among the experimental groups, Group C (Remineralised teeth with Nano Hydroxyapatite) showed a mean microhardness of 368 ± 9.66 VHN (354.2–382.4 VHN), while Group D (Remineralised teeth with CCP-ACP) recorded 319.4 ± 15.8 VHN (294.7–339.3 VHN).

The analysis of variance (ANOVA) test indicated significant differences among the study groups ($P < 0.0001$). Groups denoted with different superscript

letters (A, B, C, D) demonstrated statistically significant differences in pairwise comparisons.



NHA:Nano Hydroxyapatite,
CPP-ACP:Casein phosphopeptide–amorphous calcium phosphate

Bar Graph: Inter-group comparisons of the Microhardness test (in VHN) among the study groups

DISCUSSION

Enamel remineralisation is a dynamic biological process that restores mineral content lost during demineralisation, caused by acidic challenges in the oral environment [16]. Enamel demineralisation and remineralisation represent a continuous and dynamic equilibrium that governs the initiation, progression and possible reversal of dental caries [2]. Demineralisation occurs when organic acids produced by bacterial metabolism of fermentable carbohydrates lower the plaque pH below the critical threshold of approximately 5.5, resulting in the dissolution of calcium and phosphate ions from the enamel surface and subsurface regions [17]. Remineralisation is a physicochemical process driven by the redeposition of calcium and phosphate ions into partially demineralised enamel crystals when the pH returns to neutral or supersaturated conditions [9].

Research in enamel remineralisation is essential to address the limitations of existing therapies, reduce fluoride dependency, improve subsurface lesion repair, and align caries management with minimally invasive principles [16]. A compelling reason for ongoing research is the increasing concern regarding fluoride overexposure and dental fluorosis [18]. This has driven interest toward alternative or adjunctive remineralising systems that can reduce reliance on fluoride while maintaining or enhancing clinical efficacy [19].

In enamel remineralisation research, artificial caries lesion models are widely used to create controlled and reproducible subsurface demineralisation that mimics early carious lesions [10]. A common chemical demineralising solution consists of 2.2 mM calcium chloride ($CaCl_2$), 2.2 mM monosodium phosphate (NaH_2PO_4), and 0.05M lactic acid, typically adjusted to an acidic pH around 4.4 – 4.5 to induce mineral dissolution while preserving an intact surface layer resembling clinical white spot lesions [20]. The inclusion of calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions in demineralising solutions, more closely mirrors the ionic milieu in plaque fluid during acid challenges, where enamel dissolution is driven by a reduction in mineral saturation rather than absence of ions [4]. Lactic acid is selected as the acid challenge because it is the primary organic acid produced by cariogenic

bacteria such as *Streptococcus mutans* during carbohydrate fermentation [21]. Lactic acid effectively dissolves enamel hydroxyapatite, lowering pH below the critical dissolution threshold (~5.5), and forming enamel lactate salts that compromise enamel integrity, thus creating clinically relevant lesions. Moreover, the pH range of ~ 4.5 is chosen to maintain partial saturation with respect to hydroxyapatite, thus inducing controlled dissolution without total surface erosion [22]. This balanced dissolution recreates the early "white spot" lesion stage that is amenable to remineralisation, aligning with minimal intervention and non-invasive dentistry paradigms widely discussed in the literature [23].

Many authors have studied the remineralisation potential of enamel by application of various remineralisation agents over the past few years. Jihad Ahmed Salah et al. in 2025 evaluated the remineralisation potential of three different toothpastes – Nanohydroxyapatite toothpaste (Apagard Royal), Fluoride toothpaste (Sensodyne) and combination of nanohydroxyapatite and fluoride toothpaste (Curaprox) on artificially created enamel caries using an in-vitro model. At 2 weeks and 1 month, Nanohydroxyapatite toothpaste exhibited significantly higher Calcium (Ca) and Phosphorus(P) deposition and the highest Ca/P ratio, indicating superior remineralisation [24]. Sibel Akküç et al. in 2023 conducted a study to investigate the remineralization efficacy of three different agents – Sodium fluoride (Sensodyne Promine), CPP – ACP (GC Tooth Mousse) and Herbal agent (Agarta herbal toothpaste) on initial caries and erosive enamel lesions. After 15 days of remineralization, Sodium fluoride showed the highest mineral density of newly formed tissue followed by CPP – ACP, with herbal toothpaste showing the lowest values [25].

Microhardness testing is a widely accepted indirect method for evaluating mineral changes in dental enamel during demineralisation and remineralisation processes [10]. Vickers microhardness (VMH) testing provides a direct quantitative measure of changes in enamel mechanical properties following demineralisation and remineralisation [26]. Since enamel hardness is closely correlated with mineral content, VMH allows sensitive detection of mineral gain or loss and enables longitudinal comparison at the same specimen site using non-destructive indentation protocols [27].

Group A (intact enamel) showed the highest mean microhardness value (413.3 ± 21.0 VHN), which served as the gold standard reference. The superior hardness values observed in this group can be attributed to the preserved structural integrity of enamel, characterized by well-organized hydroxyapatite crystals with minimal porosity and intact interprismatic regions [3]. These findings are consistent with earlier studies by Featherstone et al. and Ten Cate et al. who have similarly reported significantly higher microhardness values for sound enamel when compared with demineralized or treated enamel surfaces [26].

In contrast, Group B (demineralized enamel) recorded the lowest mean microhardness value (277.6 ± 17.7 VHN). This drastic reduction in hardness reflects the loss of mineral content due to acid-induced dissolution of calcium and phosphate ions from the enamel surface and subsurface [28]. Amaechi et al., Arends and Ten Cate et al. reported similar reductions in microhardness following demineralization in their

literature, confirming that enamel softening is a sensitive indicator of early mineral loss [29,30,31].

Among the remineralizing agents tested, Group C (nanohydroxyapatite) demonstrated the highest mean microhardness value (368 ± 9.66 VHN). This superior performance may be attributed to the nano-sized particles, which closely mimic the size and morphology of natural enamel apatite crystals [32]. Nanohydroxyapatite has a high surface area and strong affinity for enamel, enabling it to penetrate subsurface lesions and act as a template for mineral deposition [33]. Additionally, it promotes crystal growth by supplying bioavailable calcium and phosphate ions, facilitating true biomimetic remineralization rather than mere surface precipitation [34]. According to Li L et al. and Huang et al., - Nanohydroxyapatite also acts as a filler because it repairs small holes and depressions on enamel surface, a function enhanced by the small size of the particles that compose it. By penetrating the enamel pores, nanohydroxyapatite crystals act as a template in the precipitation process and promote crystal integrity and growth [35].

Group D (CPP–ACP) showed a mean microhardness value of 319.4 ± 15.8 VHN. CPP–ACP acts by stabilizing calcium and phosphate ions in an amorphous form and maintaining a state of supersaturation around the enamel surface, thereby enhancing remineralization [12]. However, its mechanism relies heavily on ion availability and diffusion rather than direct crystal deposition.[36] This may explain why CPP–ACP, although effective, did not achieve hardness values comparable to nanohydroxyapatite or intact enamel.

When we look at the limitation of the study, it is evident that the present study model was conducted under *in vitro* conditions using extracted human premolars. Factors such as salivary flow and composition, pellicle formation, plaque biofilm activity, masticatory forces, and patient-related variables were not simulated. Hence, the remineralisation outcomes observed in this study may not be directly extrapolate to clinical conditions. The remineralising efficacy of the tested agents was evaluated solely using Vickers surface microhardness testing. Advanced analytical techniques such as transverse microradiography or micro-CT could have provided a more comprehensive evaluation of mineral distribution within the lesion body.

CONCLUSION

Within the limitations of our study, the results of the current investigation concluded that Nanohydroxyapatite is a better remineralising agent in comparison to CPP-ACP. Thus remineralisation procedures can be considered the most preferred and optimal way of regeneration of lost tooth structure, thereby directing the focus of restorative dentistry towards a more conservative approach.

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