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Original Article

The effect of nano-hydroxyapatite and casein phosphopeptide-amorphous calcium phosphate with and without laser irradiation on the microhardness and surface morphology of demineralized primary enamel: An *in vitro* experimental study

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ABSTRACT

Background: Various topical gels, varnishes, and fluoride gels are being used by dentists for the treatment of White spot lesions (WSLs). The remineralizing effect of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), nano-hydroxyapatite (nHAp), and lasers has been proven earlier. This study was designed to evaluate the remineralizing effect of nHAp and CPP-ACP with and without erbium-doped yttrium aluminum garnet (Er:YAG) laser irradiation on demineralized primary enamel. The aim of this study was to evaluate the effect of CPP-ACP and nHAp with and without Er:YAG laser irradiation on the microhardness and surface morphology of demineralized primary enamel.

Materials and Methods: The present study is an experimental *in vitro* study. Fifty extracted primary incisors were selected for the study. Following cleaning and sectioning, teeth were embedded in acrylic. The tooth models were divided into four groups randomly – Group 1 (CPP-ACP), Group 2 (nHAp), Group 3 (CPP-ACP + laser), and Group 4 (nHAp + laser). The baseline, postdemineralization, and postremineralization Vickers hardness testing was performed. One sample from each group was analyzed by scanning electron microscopy. Descriptive statistics such as frequencies and percentages for categorical data, mean and standard deviation for numerical data were depicted. The normality of numerical data was checked using the Shapiro–Wilk test. The level of significance was kept at 5%. Intergroup comparison (>2 groups) was done using one-way analysis of variance followed by pair-wise comparison using the *post hoc* test.

Results: There was a statistically significant increase in surface microhardness in each group after remineralization. The highest increase in microhardness value was seen in Group 4 (nHAp + laser) followed by Group 3 (CPP-ACP + laser) and the least in Group 1 (CPP-ACP). Similar observations were made in scanning electron microscopic images. This indicated that nHAp has a comparable, if not better ability for remineralization than CPP-ACP. The remineralizing capacity of both the

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remineralizing agents was seen to be improved in this study when simultaneous laser application was employed.

Conclusion: Currently, the evidence supporting the efficacy of nHAp dentifrices and laser in primary teeth is limited. Additional long-term *in vivo* studies employing standardized protocols and large sample sizes are necessary to draw definitive findings about the effect of remineralizing agents and lasers on primary enamel.

Key Words: Deciduous teeth, hydroxyapatite, laser, tooth remineralization

INTRODUCTION

The interaction of the bacteria on the tooth surface, the dental plaque or oral biofilm, the diet, and the teeth acting together over time all contribute to dental caries. Fundamentally, an imbalance between demineralization and remineralization is what causes tooth decay.[1]

Remineralization is the net gain in minerals that were previously lost due to demineralization at the surface of the enamel. Early treatment of developing carious lesions seeks to remineralize a subsurface lesion that is still active but not cavitating, preventing further mineral loss from cavitating the lesion.[1]

In the past few years, dentists have employed a variety of topical agents such as gels and varnishes, to treat WSLs. Subsurface caries tend to remineralize with these substances by giving out calcium phosphate, either with or without fluoride, and managing the microenvironment.[1]

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), nano-hydroxyapatite (nHAp), and erbium-doped yttrium aluminum garnet (Er:YAG) laser have been studied for remineralization of incipient caries in permanent teeth. However, not many studies have been done to analyze the action of Er:YAG laser in combination with nHAp and CPP-ACP for primary enamel remineralization. Such research will aid in employing lasers to improve the action of remineralizing agents. It may reduce the number of applications of such agents and the overall number of dental visits for preventive and remineralization therapy.

Hence, this study aimed to evaluate the effect of nHAp and CPP-ACP with and without Er:YAG laser irradiation on the microhardness and surface morphology of demineralized primary enamel.

METHODOLOGY

The study was conducted on primary incisor teeth collected from the Department of Pediatric and Preventive Dentistry, Bharati Vidyapeeth (Deemed to be University), Dental College and Hospital, Sangli. The study performed was an experimental *in vitro* study.

Materials

- Folowing materials [Figure 1] were used for the study-
- Extracted primary incisor teeth
- Aclaim Toothpaste (Group Pharma Ltd.) (nanoXIM•CarePaste – Fluidinova)
- GC Tooth Mousse (CPP-ACP) (Recaldent, TM, GC Company)
- Dr. Smile Er:YAG Hard Tissue Laser (PLUSER, 10 W)
- Vickers hardness tester (Omni Tech MVH-G, Pune)
- Scanning electron microscope (JEOL JSM-6360)
- 0.10% thymol solution (Sisco Research Laboratory Pvt. Ltd., Hyderabad)
- Microbrush (disposable applicator) (GC India Dental Pvt. Ltd., Telangana)
- 600 grit grinding disk (SHOFU Dental Corporation, SM, CA, USA)
- Demineralizing solution (BVDUMCH, Sangli)
- DPI-RR Cold Cure Acrylic resin (Dental Products of India)
- Acid-resistant transparent nail varnish.

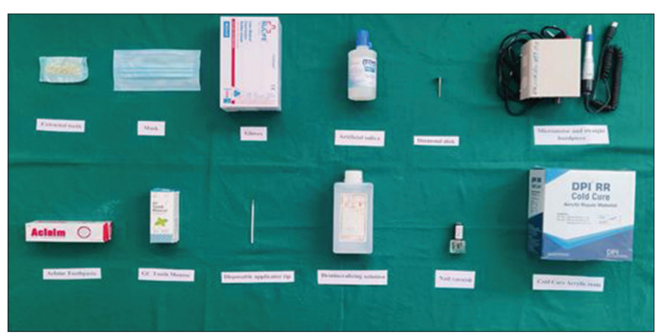


Figure 1: Armamentarium.

The number of extracted teeth (sample size) selected for the study was determined as follows:

With reference (the effect of combining laser and nHAp on the surface properties of enamel with initial defects – Assal DW – 2018)[2]

Effect size: 1.6889151

Alpha error: 0.05

Power: 0.95.

The minimum sample size calculated by using G*Power (Dusseldorf Germany. Erdfelder, Faul, and Buchner, 1996) software was 11 per group.

Therefore, 12 teeth per group were suggested.

The teeth were divided into four study groups.

Therefore, the total sample size was 48.

Two additional teeth will be used for scanning electron microscopy (SEM) (one with sound enamel and one with demineralized enamel).[1]

Therefore, the total sample size was 50.

Hence, 50 over-retained primary incisor teeth having a sound enamel surface were selected for the study. Teeth having hypo-mineralization defects, carious teeth, discolored teeth, and fractured or cracked teeth were excluded from the study [Figure 2a].

The teeth were stored in 0.1% thymol solution immediately after extraction for 24 h. The teeth were cleaned using an ultrasonic scaler [Figure 2b]. The teeth were dried and coated with acid-resistant nail varnish leaving a square window, 2 mm × 2 mm wide for demineralization on the labial surface [Figure 2c]. The teeth were sectioned 1 mm below the cementoenamel junction (CEJ) [Figure 2d and e]. To make both handling and identification easier, the teeth were mounted in acrylic resin [Figure 2f] in such a way that the facial enamel surface was exposed.

The baseline surface microhardness of sound enamel was checked using a Vickers microhardness tester. Surface microhardness was measured using the Vickers diamond microindenter. A force of 100 g for 15 s was applied to the enamel surface by a diamond instrument at three points. Reading was obtained as Vickers hardness number.

Artificial carious lesions were formed on each enamel specimen by placing them in a 50 ml demineralizing solution (2.2 Mm $CaCl_2.2H_2O$, 2.2 Mm $NaHPO_4.7H_2O$, 0.05 M lactic acid) at 37°C temperature in an incubator for 48 hours.[3]

After washing with distilled water and drying, all the samples were subjected to postdemineralization microhardness testing using a Vickers tester.

The specimens were divided into four groups randomly.

- Group 1 – CPP-ACP (GC Tooth Mousse – Recaldent, TM, GC Company) paste was applied to the specimen using a microbrush and kept for 3 min (according to manufacturer's instructions) followed by pH cycling [Figure 3a]
- Group 2 – nHAp paste (Aclaim Toothpaste – Group Pharma Ltd. – nanoXIM•CarePaste) was applied to the specimen using a microbrush and

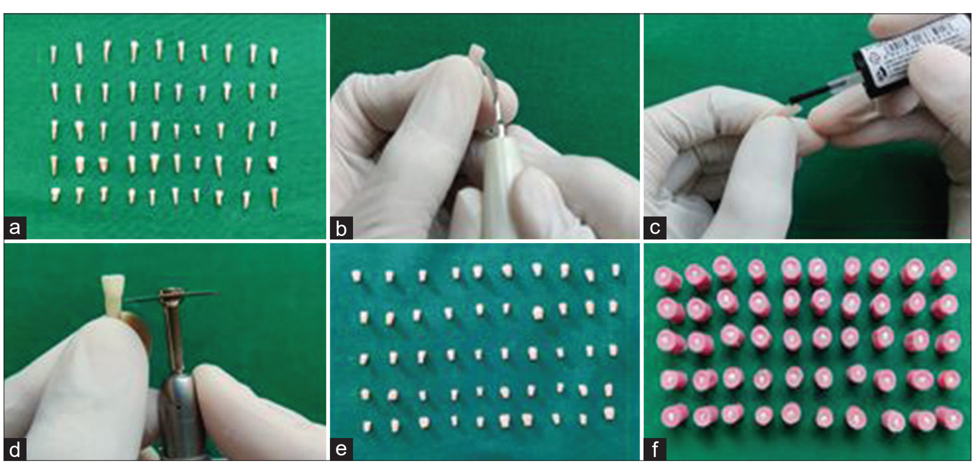


Figure 2: Preparation of tooth models. (a) Primary incisors selected for the study, (b) Incisor cleaned using ultrasonic scaler, (c) Nail varnish applied on labial surface, (d) Root sectioned 1 mm below the cementoenamel junction, (e) Sectioned teeth, (f) Acrylic tooth models.

Figure 3: Remineralization of enamel surface. (a) Application of casein phosphopeptide-amorphous calcium phosphate paste in models of Group 1, (b) Application of nano-hydroxyapatite paste in models of Group 2, (c) Laser irradiation of tooth models in Groups 3 and 4 followed by respective paste application.

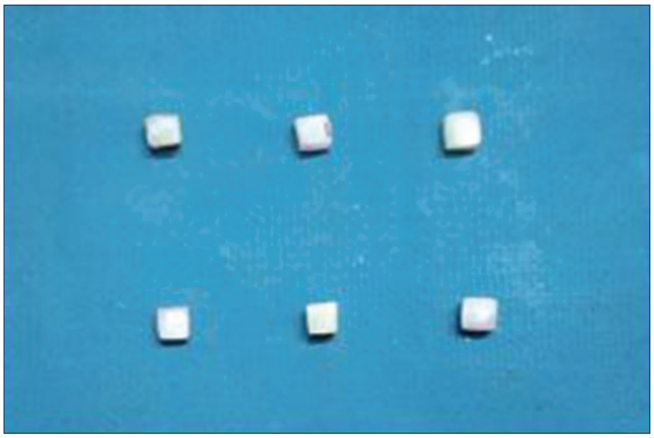


Figure 4: Tooth specimens modified for scanning electron microscopy.

kept for 3 min (according to manufacturer's instructions) followed by pH cycling [Figure 3b]

- Group 3 – Laser irradiation (Dr. Smile Er:YAG Hard Tissue Laser – PLUSER -10 W) was followed by CPP-ACP paste application [Figure 3c]
- Group 4 – Laser irradiation was followed by nHAp paste application [Figure 3c].

The Er:YAG laser emitting at a wavelength of 2.94 μm was used to irradiate the exposed enamel. The parameters were 80 mJ of energy and a 4 Hz frequency for 10s and energy per pulse of 80 mJ with water spray and 1.2 mm spot size.

Following remineralization, pH cycling was done for 15 days. On the 15th day, the surface microhardness of the remineralized specimen was checked using a Vickers tester.

SEM (JEOL JSM-6360) was carried out for one representative sample per group ($n = 4$) and two additional samples:

a. One sample of sound enamel
b. One sample of demineralized enamel.[1]

Therefore, the total number of the specimen subjected to SEM was,

$n = 4 + 2 = 6$.

The acrylic rods were trimmed to form cubes of 3 mm^3 [Figure 4] to fit onto the specimen grid for viewing.[4]

The enamel surface was coated with a thin layer (2–10 nm) of gold (gold sputtering) right before observing the specimen under the scanning electron microscope.

Statistical analysis

Data obtained were compiled on MS Office Excel Sheet (v 2010, Microsoft Redmond Campus, Redmond, Washington, United States). Data were subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS version-19.0, International Business Machines, Chicago, United States of America). Descriptive statistics such as frequencies and percentages for categorical data and mean and standard deviation for numerical data were depicted. The normality of numerical data was checked using the Shapiro–Wilk test and was found that the data followed a normal curve; hence, parametric tests were used for comparisons. Intergroup comparison (>2 groups) was done using one-way analysis of variance (ANOVA) followed by pair-wise comparison using the *post hoc* test.

For all the statistical tests, $P < 0.05$ was considered to be statistically significant, keeping α error at 0.01 and β error at 20%, thus giving power to the study as 99%. The level of significance was kept at 5%.

RESULTS

Table 1 shows the comparison of microhardness at baseline.

The results revealed that there was a nonsignificant difference in microhardness of the four groups at baseline.

Table 2 shows the comparison of microhardness before and after demineralization in each group.

There was a significant reduction in the microhardness of each group after demineralization.

Table 3 shows the comparison of microhardness before and after remineralization in each group.

The results of Table 3 and Graph 1 showed that there was a nonsignificant difference in the microhardness of the four groups after remineralization.

Table 4 shows the comparison of the percentage increase in the microhardness of four groups after remineralization.

According to Table 4 and Graph 2, the highest increase was seen in Group 4 followed by Group 3 and the least in Group 1. However, there was a nonsignificant difference in the percentage increase in the microhardness of the four groups after remineralization.

SEM images revealed the following findings:

For sound enamel surface [Figure 5a], normal, smooth, and intact enamel surface structure devoid of any alteration was noted before demineralization; normal perikymata appeared to run in parallel lines. Some porosities could be seen.

The structure of the enamel surface after demineralization is changed in Figure 5b. Enamel lost its normal architecture and the prisms showed irregularity. Numerous voids and microporosities were seen on the enamel surface.

In Figure 5c, the changes in enamel surface structure after treatment with CPP-ACP (Group 1)

Table 1: Comparison of microhardness at baseline (Vickers hardness number)

Group	Mean (VHN)	SD	*P*	Pairwise comparison
Group 1	295.40	8.44	0.088	Group 1 versus Group 2, Group 3, Group 4
Group 2	299.18	4.83		Group 2 versus Group 3, Group 4
Group 3	297.79	5.07		Group 3 versus Group 4
Group 4	301.23	1.87		

One-way ANOVA test; *Post hoc* Tukey's test. SD: Standard deviation; VHN: Vickers hardness number

Table 2: Comparison of microhardness after demineralization (Vickers hardness number)

Group	Mean (VHN)	SD	*P*	Pairwise comparison
Group 1	181.76	5.40	0.004*	Group 1 >Group 2, Group 4; Group 1 versus Group 3
Group 2	176.12	3.82		Group 2 versus Group 3, Group 4
Group 3	177.01	4.59		Group 3 versus Group 4
Group 4	175.08	4.09		

*Significant difference at $P \leq 0.05$. One-way ANOVA test; *Post hoc* Tukey's test. SD: Standard deviation; VHN: Vickers hardness number

Table 3: Comparison of microhardness after remineralization (Vickers hardness number)

Group	Mean (VHN)	SD	*P*	Pairwise comparison
Group 1	265.85	12.87	0.128	Group 1 versus Group 2, Group 3, Group 4
Group 2	258.59	10.12		Group 2 versus Group 3, Group 4
Group 3	264.03	11.19		Group 3 versus Group 4
Group 4	269.72	10.95		

One-way ANOVA test; Post hoc Tukey's test. SD: Standard deviation; VHN: Vickers hardness number

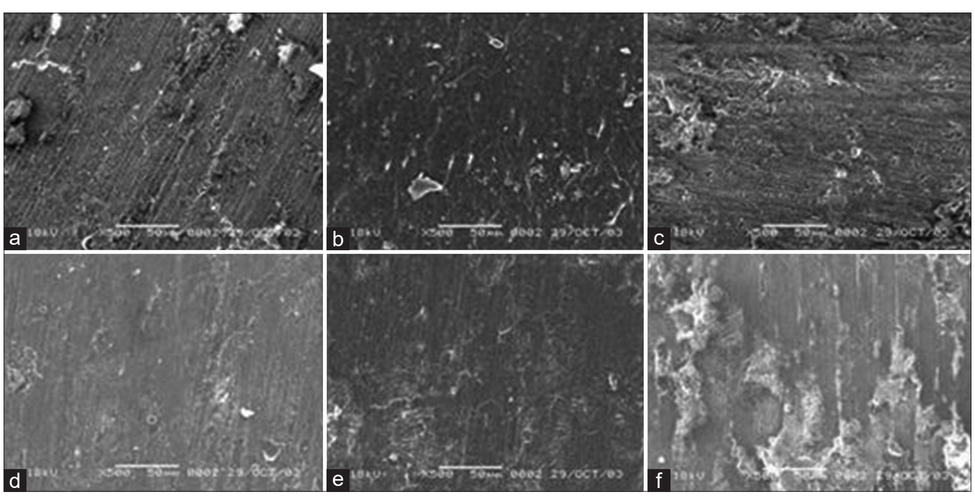
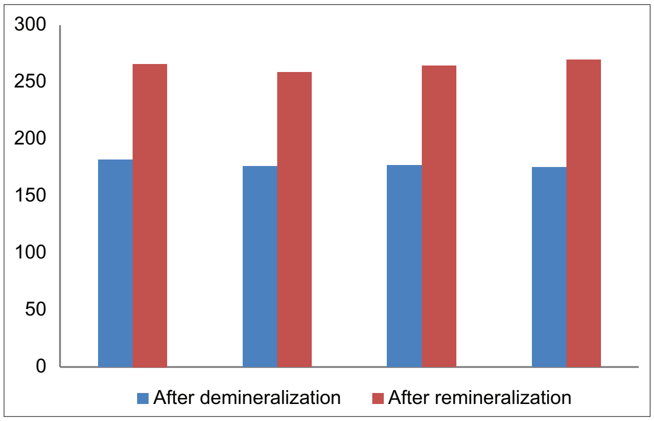
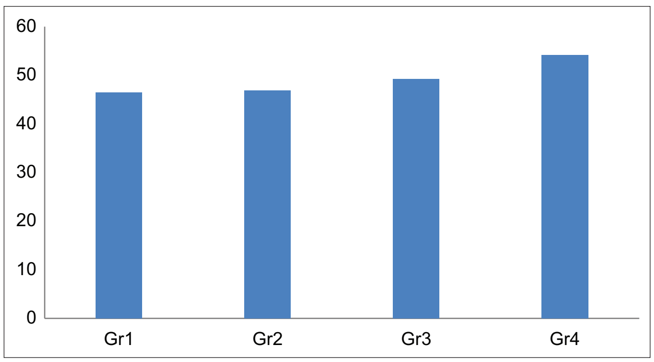


Figure 5: Scanning electron microscopic images. (a) Sound enamel. (b) Demineralized enamel. (c) Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) paste applied on enamel, (d) Nano-hydroxyapatite paste applied on enamel, (e) Laser irradiation followed by CPP-ACP paste application on enamel, (f) Laser irradiation followed by nano-hydroxyapatite paste application on enamel.



Graph 1: Comparison of the microhardness before and after remineralization in each group.



Graph 2: Comparison of the percentage increase in microhardness after remineralization.

were demonstrated. Morphological changes were observed by the presence of amorphous, globular, and crystalline structures that occlude the micropores created after demineralization.

In Figure 5d, most of the microporosities were hidden and occluded. The nHAp particles (Group 2) appeared to be melted, recrystallized, and trapped inside the pores forming a plug.

Figure 5e and f shows the SEM image of the enamel surface irradiated with Er:YAG (Group 3, 4) laser. It showed an absence of normal enamel perikymata, cracks, craters, etc. Irregular melted and recrystallized areas were seen on the enamel surface.

The SEM image of the enamel surface treated with Er:YAG laser + CPP-ACP (Group 3) in Figure 5e shows that the interprismatic substances were evident, with porosities and areas of remineralization. It also displayed thick and more frequent lines of remineralization along the prismatic borders; certain areas of calcifications were evident along the porosities.

Table 4: Comparison of % increase in microhardness after remineralization

Group	Mean	SD	*P*	Pairwise comparison
Group 1	46.43	9.36	0.102	Group 1 versus Group 2, Group 3, Group 4
Group 2	46.90	7.02		Group 2 versus Group 3, Group 4
Group 3	49.29	8.10		Group 3 versus Group 4
Group 4	54.19	8.55		

One-way ANOVA test; *Post hoc* Tukey's test. SD: Standard deviation; VHN: Vickers hardness number

The SEM image of the enamel surface treated with Er:YAG laser + nHAp (Group 4) in Figure 5f showed that the surface defects that were produced by laser irradiation were reconstructed by the deposition of a layer of nHAp particles.

DISCUSSION

The enamel must endure a variety of physical and chemical processes, such as compressive stresses (up to around 700N), physiologic and pathologic wear of tooth structure, and most critically, an acidic environment brought on by the presence of bacterial plaque and food. Due to their proximity to saliva and salivary plaque, the HAp crystals near the enamel's surface are always active. The process of demineralization takes place at pH 5.5, where HAp dissolves as a result of acid generated by bacterial metabolic products or by intake of acidic foods.[5]

If allowed to continue for a long time, significant mineral loss results in enamel loss and subsequent cavitation. Deposition of calcium, phosphate, and fluoride ions (fluorapatite) improves resistance to dissolution by organic acids as fluorapatite crystals have an advancing growth pattern, leading to the formation of large crystals with hexagonal outlines. The demineralization process continues until the pH rises, causing remineralization to occur. Therefore, improving the remineralizing process with the use of remineralization products is the greatest technique for caries control.

The early demineralization of the enamel at the surface and subsurface is known as a WSL, which manifests as a milky white opacity. If left untreated, subsurface demineralization increases porosity, which eventually alters the optical characteristics of the teeth and causes cavitation.[5]

The use of fluoride dentifrice to regularly brush teeth has been advised for years, however using fluoride continuously at higher than optimal doses has been linked to fluorosis and fluoride poisoning in children, especially those under the age of six. Additionally, a layer that resists acid formation may develop, which hinders the diffusion of remineralizing ions into deeper layers and restricts overall remineralization of the region.[6]

A potential alternative remineralizing agent with anti-caries properties has been identified based on calcium phosphate. It is CPP-ACP. In low-pH environments, it has been demonstrated to prevent demineralization by releasing calcium and phosphate ions.[7]

As a result of stabilizing calcium phosphate in solution by binding ACP to numerous phosphoserine residues and forming small clusters of CPP-ACP, the anticariogenic effects of CPP-ACP are related to increasing the buffering effect of saliva, which suppresses demineralization and activates remineralization, or both.[7]

A biocompatible synthetic substance called nHAp resembles the hydroxyapatite crystals found in human teeth. Its application in numerous curative, reparative, and regenerative therapies has drawn a lot of attention recently.

HA crystals with a size range of 20–40 nm make up the majority of the material in tooth enamel. Due to the structural and chemical similarities between nano-sized HA crystals and enamel apatite crystals, synthetic nHAp is used for remineralization applications. Additionally, nHAp has been demonstrated to have a remineralizing impact on artificial carious lesions and develop a new enamel layer. It is more biocompatible than HA and has stronger bioactivity and mechanical characteristics.[8]

Numerous studies conducted over the past four decades have demonstrated the use of laser irradiation on dental tissues in preventing enamel erosion or WSLs. When teeth are exposed to the laser, the light and the microscopic components of the dental hard tissue interact. The irradiation energy changes into heat instantly when the particular components absorb the light. Enamel is thought to undergo microstructural and chemical changes as a result of heat, which also accounts for its greater acid resistance.[7]

In the current investigation, nail polish was applied to the primary incisors' labial surface, leaving a 2 * 2 mm window. The teeth were sectioned 1 mm below the CEJ and embedded in self-cure acrylic to form tooth models. Each enamel specimen underwent a 48-h demineralization simulation in a 50 ml demineralizing solution made up of 2.2 Mm CaCl2.2H2O, 2.2 Mm NaHPO4.7H2O, and 0.05 M Lactic acid at 37°C in an incubator. This created artificial carious lesions on the enamel samples. All of the samples were tested for postdemineralization microhardness using Vickers testers after being rinsed with distilled water and dried.

In a study by Nair *et al*. in 2016, the specimens were individually submerged in 5 ml of an acetate buffer solution (0.1 mol/L, pH 4.5) and incubated at 37°C for 24 h to mimic the oral environment.[9] A hundred specimens were prepared from 50 human premolars to explore the caries-inhibiting effects of remineralizing agents and laser on enamel using an atomic emission spectrometry analysis. The enamel samples were divided into 6 groups at random, including Untreated (Control), CPP-ACP (GC Tooth Mousse), CPPACFP (GC Tooth Mousse Plus), Er:YAG laser therapy alone, CPP-ACP with Er:YAG laser, and CPP-ACFP with Er:YAG laser. They came to the conclusion that, as compared to other groups, the combination of CPP-ACFP and Er:YAG laser was more effective at reducing enamel demineralization.

In the present study, the specimens of Group 1 were treated with CPP-ACP paste. The paste was applied on the surface of the specimen using a disposable micro applicator tip and kept for 3 min, followed by pH cycling. In a study by Oshiro and Yamaguchi, two sets of specimens were placed in 10-fold diluted CPP-ACP paste (Tooth Mousse, GC Corp. Tokyo, Japan) or placebo paste (without CPP-ACP) for 10 min each before being placed in a demineralizing solution.[10] In a vacuum evaporator, a small layer of gold was applied to the surfaces, and they were then examined using field emission SEM. CPP-ACP paste treatment caused minor morphological alterations in enamel and dentin specimens. It was concluded that CPP-ACP paste may prevent the demineralization of the tooth structure, according to the morphological observations of the enamel and dentin surfaces. The results of the present study demonstrated a significant increase (<0.001) in the microhardness of Group 1 after remineralization (265.85) in comparison to postdemineralization values (181.76).

In group 2, the specimens were treated with nHAp paste. The paste was applied on the surface of the specimen using a disposable micro applicator tip and kept for 3 min, followed by pH cycling. The impact of nHAp and CPP-ACP fluoride paste on artificial enamel carious lesions of young permanent teeth was compared by Abdelaziz *et al*. in 2019.[11] For the investigation, 60 extracted premolar teeth were chosen. The enamel surface microhardness was assessed at baseline, after the formation of an enamel lesion, and following therapy. They came to the conclusion that both CPP-ACP fluoride and nHAp were successful at remineralizing young permanent teeth with early caries-like lesions. The present study showed similar findings, where postdemineralization values of Group 3 specimens (176.12) showed a significant increase (<0.001) in the postremineralization values (258.59).

For the specimen in Group 3, the CPP-ACP paste was applied after laser irradiation. The Er: YAG LASER emitting at a wavelength of 2.94 micrometers was used to irradiate the exposed enamel. The parameters were: 80 mJ of energy and a 4 Hz frequency for 10 s and energy per pulse of 80 mJ with water spray and 1.2 mm spot size. The results of the present study showed a significant (<0.001) rise in the microhardness values of this group after remineralization.

For their investigation, Khamverdi *et al*. used thirty sound maxillary extracted premolars.[12] The crowns were divided into labial and palatal portions at the cervical line. Specimens were mounted in acrylic blocks. The samples underwent pH cycling before being randomly divided into 4 groups (n = 15), as follows: CG stands for the control group, LAS for CO2, CP for CPP-ACP, and LASCP for laser-combined CPP-ACP therapy. The demineralized enamel surface was treated with CPP-ACP paste for 5 min, followed by washing. The surface of the specimen was irradiated with a CO2 laser at a wavelength of 10.6 μm for LAS and LASCP. Following were the laser's settings: power of 0.7 W, 50 Hz pulse frequency, 0.2 mm focal spot, 0.4 msond pulse length, noncontact mode, 10 mm between hollow tube tip and tooth surface, and spot size: 0.4 mm. The Vickers microhardness of each sample was determined. According to their findings, the CO2 laser and CCP-ACP were useful for increasing enamel hardness following demineralization. The application of CCP-ACP paste and CO_2 laser irradiation increased the demineralized enamel's ability to remineralize.

In the present study, for the specimen of Group 4 nHAp paste was applied after laser irradiation. The results of the present study demonstrated a significant increase (<0.001) in the microhardness of Group 4 after remineralization (269.72) in comparison to postdemineralization values (175.08).

Assal *et al*., prepared pure hydroxyapatite (nHA) and fluoro hydroxyapatite (nFHA), two different forms of nHAp.[2] They used sixty extracted premolar teeth which were demineralized. They were then divided into two groups at random – Group 1: nHA, Group 2: nFHA – and then into two subgroups (A and B), each of which underwent two distinct *in vitro* remineralization procedures. The first procedure only used 10-weight percent nHA aqueous slurries, while the second involved first being exposed to fractional CO_2 laser irradiation before nHAp was applied. A micro-Vickers hardness tester and a spectrophotometer were used to quantify microhardness and color, respectively. They came to the conclusion that nHAp has exceptional remineralizing effects on early enamel lesions, effects that are undoubtedly enhanced by the addition of laser therapy.

Amaechi *et al*. evaluated how two kinds of toothpaste with hydroxyapatite or 500 ppm fluoride promoted remineralization and prevented tooth decay.[13] In a two-arm, double-blind, randomized crossover study, 30 adults wore intraoral appliances for 14 days each, during which time they were exposed to toothpaste containing either 10% hydroxyapatite or 500 ppm F (amine fluoride), depending on which enamel block had an artificially-produced caries lesion. Lesion depth and baseline and posttest mineral loss were measured using microradiography. They found that 10% hydroxyapatite was as effective as 500 ppm F in remineralizing early caries.

Additional remineralizing and preventive agents are frequently required to enhance caries preventive effect of fluoride in people with high caries risk. The fluoride dose recommended for toddlers and children is even lower than the regulatory 1000–1500 ppm fluoride concentration in nonprescription toothpaste, which is probably suboptimal for effective remineralization of initial lesions. An agent that is as effective as fluoride but whose dosage may be increased without raising any safety issues is anticipated to be a better option, particularly for kids.[13]

Nourolahian *et al*. compared the remineralization efficacy of Remin Pro (HAp, fluoride, and xylitol) with CPP-ACP and acidulated phosphate fluoride gel. In their study, 96 enamel samples were prepared. Baseline microhardness was measured for all the samples using the Vickers microhardness test. After developing the initial caries lesions, the microhardness of all the demineralized samples was measured, and the samples were then divided into four groups (n = 24). CPP-ACP in Group 1, Remin Pro in Group 2, and APF gel in Group 3 were placed on the samples for 4 min. The control group received no treatments. The microhardness of the samples was measured again following a pH cycle of 5 days. The data were analyzed by ANOVA and the *post hoc* test at the significance level of $P < 0.05$. They reached the conclusion that APF, CPP-ACP, and Remin Pro showed the same remineralization capacity for increasing demineralized enamel hardness.

The present study, along with other studies in the past decade, highlights the efficacy of nonfluoridated agents and dentifrices in caries control. Pediatric and general dentists should strive to learn about such newer remineralizing agents and employ them in their practice.

The limitations of the present study were the need for a larger sample size and a longer observation period after the intervention.

CONCLUSION

Dentistry in the 21st century is rapidly changing with innovations in technology and the development of newer dental materials. Given the unique role of the enamel surface layer in the progress of dental caries, the assessment of changes in this region is imperative.[14] New improved materials capable of enamel remineralization even in low salivary calcium and phosphate ion concentrations are the need of the hour. Some of them are bioactive glass, CPP-ACP, Tri-calcium phosphate, etc.

Considering the results of this *in vitro* study, it can be assumed that nHAp has a comparable, if not better ability to remineralize primary enamel when compared to CPP-ACP. It was also observed that Er: YAG laser irradiation improved the remineralizing capacity of both the agents used in the study.

Further studies should be conducted *in vivo* for closer simulation of the oral environment and also by using newer methods of imaging such as FEM and spectrophotometry.

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Conflicts of interest
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