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Comparative evaluation of remineralizing potential of galla chinensis with nanohydroxyapatite, chicken egg shell and fish scale derived nanohydroxyapatite on early enamel caries- A single-blinded *In vitro* study

Bazila Malik [a], Sonali Taneja [a,*], Ramesh Bharti [b], Abhik Mukherjee [a]

[a] Department of Conservative Dentistry & Endodontics, ITS Centre for Dental Studies and Research, Ghaziabad, Uttar Pradesh, India
[b] Department of Conservative Dentistry and Endodontics, Faculty of Dental Sciences, King George's Medical University, Lucknow, India

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ABSTRACT

Objective(s): This study aimed to evaluate and compare the remineralization potential of plant-based Galla chinensis extract (GCE) with nanohydroxyapatite (nHAp), chicken eggshell-derived nHAp (CES nHAp), and fish scale-derived nHAp (FS nHAp), against casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF), on early artificial enamel lesions in human premolars.

Materials and methods: A total of 148 human premolar specimens were prepared and demineralized to simulate early enamel caries, with five intact samples serving as baseline controls. Among these, 48 samples underwent surface microhardness testing using the Vickers hardness test, 60 were analyzed for lesion depth using Polarized Light Microscopy (PLM), 30 for elemental composition using Energy Dispersive X-ray (EDX) spectroscopy, and 10 for surface morphology using Scanning Electron Microscopy (SEM). The specimens were randomly assigned into five groups: demineralized control, CPP-ACPF, GCE with nHAp, CES nHAp, and FS nHAp. A seven-day pH cycling regimen simulated the oral environment. Microhardness values before demineralization (SMH1), after demineralization (SMH2), and after remineralization (SMH3) were recorded to calculate percentage surface microhardness recovery (%SMHR).

Results: The GCE nHAp group exhibited the highest %SMHR and the shallowest lesion depth, with EDX confirming a significantly higher calcium-to-phosphorus ratio. SEM images revealed smoother, more uniform surfaces in this group. CES nHAp and CPP-ACPF demonstrated moderate remineralizing effects, while FS nHAp showed the least efficacy, with no significant difference from CPP-ACPF.

Conclusion(s): GCE combined with nHAp was the most effective remineralizing agent, followed by CES nHAp and CPP-ACPF. These findings suggest that natural plant- and animal-derived biomaterials offer promising, biocompatible alternatives for non-invasive enamel caries management.

1. Introduction

Dental caries is a multifactorial, biofilm-mediated disease characterized by enamel demineralization under acidic conditions produced by bacterial metabolites.[1] Enamel, primarily composed of hydroxyapatite crystals, remains in equilibrium with saliva at neutral pH.[2,3] A drop in pH below 5.5 disrupts the crystal lattice, leading to mineral loss and enamel breakdown. Remineralization occurs when pH is restored and calcium and phosphate ions from external sources facilitate hydroxyapatite reformation.[4] When demineralization outweighs remineralization, early enamel caries develops and may progress to cavitation if untreated.[5]

Fluoride is a well-established remineralizing agent that enhances enamel resistance by forming fluorapatite, which is less soluble in acidic environments.[6] However, fluoride alone is insufficient for complete remineralization, as adequate calcium and phosphate ions are essential.[7] Moreover, excessive fluoride exposure can result in fluorosis and systemic concerns. These limitations have driven interest in alternative agents such as casein phosphopeptide–amorphous calcium phosphate (CPP-ACP), which supplies bioavailable calcium and phosphate ions.[8] The combination of CPP-ACP with fluoride (CPP-ACPF) has shown superior remineralization and iscan be regarded as a gold standard.[9]

Biomimetic materials, particularly nano-hydroxyapatite (nHAp), have gained attention due to their structural and chemical similarity to

* Corresponding author. ITS CDSR, Delhi Meerut road, Ghaziabad, 201206, India.
E-mail address: sonalitaneja@its.edu.in (S. Taneja).

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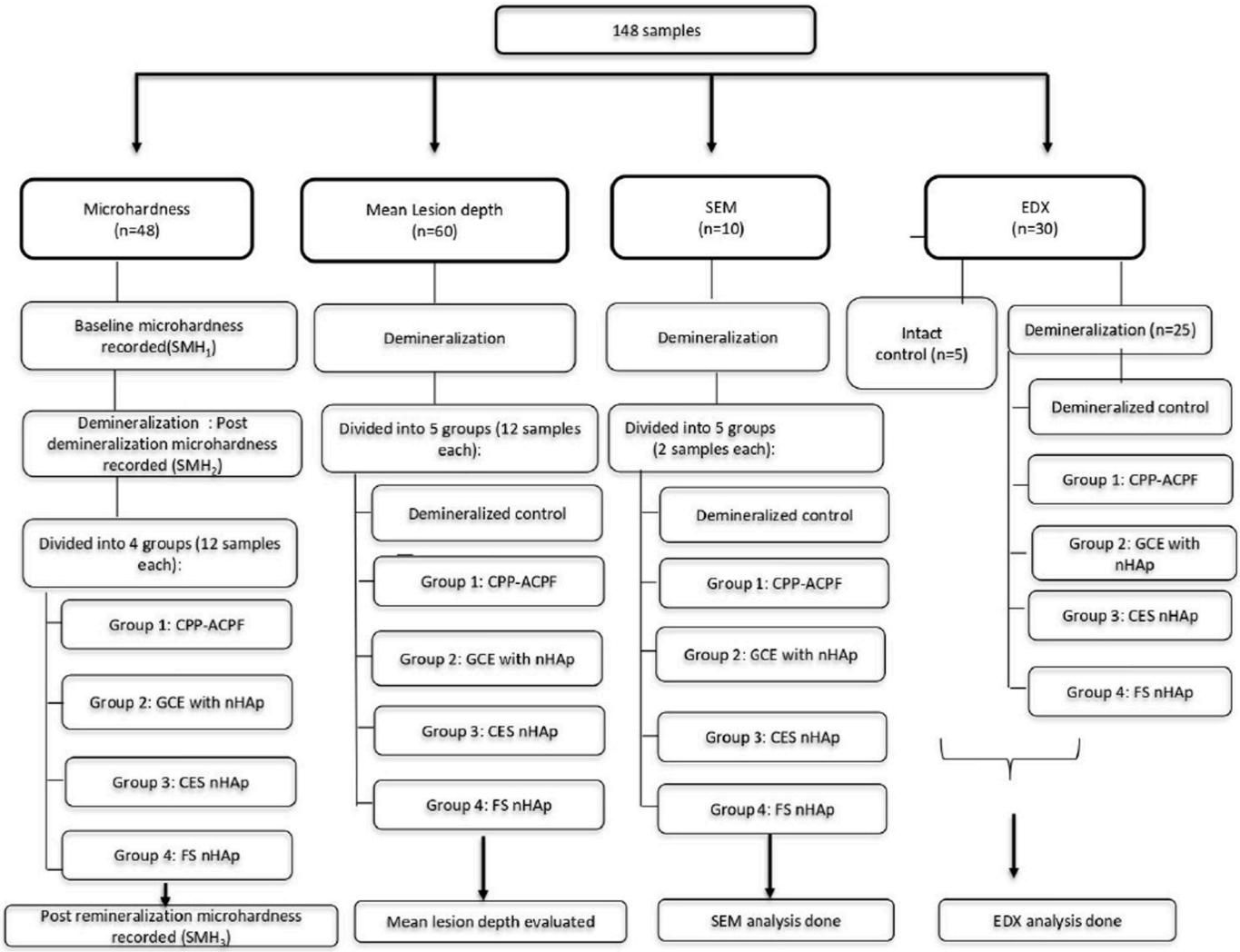

Fig. 1. Grouping of samples.

enamel.[10] Nano-hydroxyapatite particles (nHAp) exhibit enhanced solubility, effective defect filling, and promote compact enamel surface formation.[11] Sustainable plant- and animal-derived remineralizing agents have also been explored. Galla chinensis extract (GCE), a traditional Chinese herbal medicine, has demonstrated inhibition of demineralization and enhanced mineral deposition.[12,13] Animal-derived sources such as chicken eggshells and fish scales, rich in hydroxyapatite and calcium, provide biocompatible and biodegradable alternatives.[14] Eggshell-derived nHA can infiltrate enamel porosities, while fish scales, containing collagen and hydroxyapatite, serve as valuable biomaterials for enamel remineralization.[15,16]

To the best of our knowledge, no studies have compared the remineralizing efficacy of plant-based agents and animal-derived nanohydroxyapatite in reversing early enamel caries. Therefore, this study aimed to evaluate the remineralizing potential of Galla chinensis with nanohydroxyapatite, chicken eggshell-derived nanohydroxyapatite, and fish scale-derived nanohydroxyapatite in comparison with CPP-ACPF. The first null hypothesis was that there would be no significant difference in microhardness recovery among the tested remineralizing agents. The second null hypothesis stated that there would be no significant difference in lesion depth following remineralization. The third null hypothesis proposed that there would be no significant difference in the Ca/P ratio of early enamel caries treated with the different remineralizing agents.

2. Materials & methodology

2.1. Specimen collection

This study was conducted in the Department of Conservative Dentistry & Endodontics in collaboration with Centre for Advanced Research at I.T.S Centre for Dental Studies & Research, Ghaziabad and KIET School of Pharmacy, Ghaziabad. The protocol was reviewed and approved by the institutional ethical clearance committee under protocol number ITSCDSR/IIEC/LD/CONS/2022-25/002.

2.2. Specimen size determination

The sample size was calculated using the sample size calculator software program G. Power (version 3.1.9.7). The sample size calculation was based on 95% confidence interval and a power of 90%. The minimum sample size estimated for this study was one hundred forty-eight.

2.3. Specimen selection

With the patient's consent, one hundred forty-eight human maxillary premolars extracted for orthodonticreasons were collected for the study. The inclusion criteria included non-carious, intact crowns and teeth free from restorations. The samples were examined under an operating

microscope (10x) to ensure there were no cracks, fractures, or anatomical developmental anomalies. The teeth were then disinfected by autoclaving at 121 °C at 15 lbs psi and stored in freshly prepared artificial saliva Fusayama artificial saliva described by Olssom et al.[17] containing NaCl (6.8 mmol/L), KCl (5.4 mmol/L), $CaCl_2.2H_2O$ (2.7 mmol/L), $NaH_2PO4.H_2O$ (5.0 mmol/L), $Na_2S.9H_2O$ (21 μmol/L), and Urea (16.7 mmol/L)[18] titrated to pH 7.0 with NaOH until further use.

2.4. Specimen preparation

The crowns were detached from the roots using a diamond disc on a slow-speed handpiece and then divided into two halves along the mesio-distal axis to obtain buccal enamel samples (N = 148). The samples were embedded in acrylic resin with the buccal surface facing upward, and the enamel was sequentially polished using silicon carbide abrasive papers with grit sizes 1000, 600, and 320 to create flat surfaces. A 3x3 wax sheet was applied to the buccal surfaces to form a window, while the rest of the enamel was coated with acid-resistant nail varnish.[19]

2.5. Grouping of samples

The prepared enamel specimens were randomly allocated to the experimental groups using a simple randomization method to ensure equal and unbiased distribution among the groups.

Out of a total of 148 samples, 48 were assigned for microhardness testing using Vicker's Microhardness tester, 60 for mean lesion depth using PLM, 10 for SEM analysis and 30 for EDX analysis (Fig. 1).

2.6. Microhardness testing

Forty-eight samples were used for microhardness testing. A Vickers hardness tester (Banbros, India) with a diamond indenter was used to measure the baseline surface microhardness (SMH_1) of all 48 samples, applying a 500 g load for 15 s.[20] The samples were then demineralized to mimic early enamel caries using a solution containing 2.2 mM calcium chloride, 2.2 mM monosodium phosphate, and 0.05 M lactic acid. The pH was adjusted to 4.5 with 50% sodium hydroxide and confirmed using a calibrated digital pH meter.[21] Samples were immersed in this demineralizing solution in glass containers and incubated at 37 °C for 96 h. The solution was regularly replaced to maintain pH and prevent mineral buildup. After 96 h, samples were rinsed with deionized water for 30 s, air-dried for 5 s, and stored in sterile containers. Microhardness was then re-evaluated to obtain the post-demineralization value (SMH_2). Following this, the remineralization was done according to the assigned groups.

2.7. Preparation of tested remineralizing agents

I) CPP-ACPF

The commercially available GC Tooth Mousse Plus® was used.

II) GCE with nHAp

10 g of commercially available nHAp powder (Vedayukt India Private Limited) was mixed with 90 ml of distilled water and then water was added until the final volume was 100 ml to obtain 10% aqueous slurry of nHAp. 4000 ppm GCE aqueous solution was made from GCE powder (BulkSupplements.com). Equal proportion of 10% nHAp and GCE solution was mixed with each other.[22]

III) CES nHAp

Eggshells from a local hatchery were cleaned, dried, ground, and sieved. The powder was ball-milled for 5 days, and nanoparticles were formed through a precipitation reaction:

$$CaCO_3 + 2HCl \rightarrow CaCl_2 + CO_2 + H_2O.$$

The solution was mixed with water and surfactant, then centrifuged. The particles were dried and then used to prepare a 10% aqueous slurry of CES nHApby mixing with distilled water followed by sonication using ultrasonic heater.[23]

IV) FS nHAp

Fish scales from *Labeorohita* were sourced from a local market, washed, soaked in hydrochloric acid (HCl) to remove impurities, then dried. The scales were ground into particles, heated in a furnace, and wet-ground using ball milling for 48 h. The resulting slurry was dried using a spray dryer to obtain FS nHAp powder.[16] A 10% aqueous slurry of FS nHAp was then made by mixing with distilled water followed by sonication.

2.8. pH assessment

The pH of all experimental groups was measured using a calibrated digital pH meter (BOECO, Germany). Approximately 50 mL of each experimental solution was transferred into a clean beaker, and pH readings were recorded and was found to be alkaline for all the groups.

2.9. Nano particle size characterization

Nano particle size distribution for all nano particle groups was assessed using Dynamic Light Scattering (DLS). and results were expressed as volume-based size distribution, along with Z-average size and polydispersity index (PDI) to evaluate particle size and dispersion.

2.10. The pH cycling model

0.1M tris buffer (hydroxymethyl aminomethane), 0.05 ppm fluoride in the form of NaF, 0.9 mM phosphorus in the form of KH_2PO4, and 1.5 mM calcium in the form of $Ca(NO_3)_2$ with a pH of 7 was used to prepare the remineralizing solution. In the pH cycling model, the remineralizing agent from respective groups was applied to the samples for 5 min, followed by a 5-s rinse with deionized water. To mimic the daily acidic challenges in the oral environment, the teeth were then immersed in the demineralizing solution for 3 h. Then the samples were placed in the prepared remineralizing solution for the remaining 21 h of the 24-h cycle. This process was repeated daily for 7 days, with the demineralizing and remineralizing solutions being replaced every 3 days.[24]

After remineralization, microhardness was again evaluated to obtain post-remineralization surface microhardness (SMH_3) in a similar manner.

The percentage surface microhardness recovery (%SMHR) was recorded[25]

The formula used was:

$$\%SMHR = (SMH_3 - SMH_2) / (SMH_1 - SMH_2) \times 100\%$$

2.11. Mean depth of lesion

Sixty samples were used to evaluate the mean depth of enamel lesions. Demineralization was carried out as previously described. Twelve of the demineralized samples (demineralized control) were assessed for mean lesion depth using morphometric analysis and examined under polarized light microscopy (PLM). For this, the buccal surfaces were sectioned longitudinally into mesial and distal halves and ground to a thickness of 100–150 μm. After rinsing, the sections were placed on slides and viewed under PLM at 40x magnification. Photomicrographs were taken, and the demineralized areas were measured three times using ImageJ software to determine the mean lesion depth for each sample.[26,27] The remaining 48 samples were divided into four

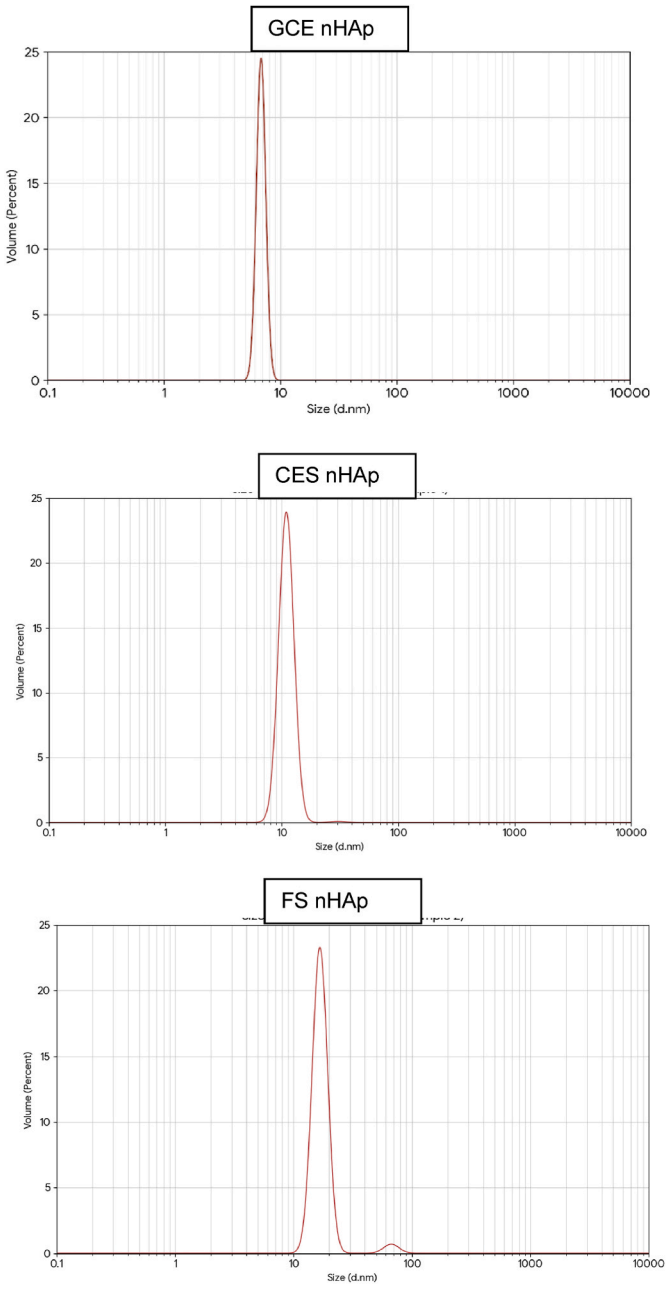

Fig. 2. Nanoparticle characterization using Dynamic Light Scattering.

Table 1
Intergroup comparison of % Surface Microhardness Recovery.

Group	Mean ± SD	95% CI for Mean	
		Lower	Upper
CPP-ACPF	32.40 ± 2.81[a]	30.62	34.18
GCE nHAp	45.63 ± 4.04	43.06	48.19
CES nHAp	36.39 ± 3.94	33.89	38.90
FS nHAp	30.49 ± 2.70[a]	28.78	32.21

One-way ANOVA; Post hoc Tukey test.
Same superscript in lowercase indicates non-significant difference among the groups with $p \leq 0.05$ considered as statistically significant.

remineralization groups of twelve and treated with different remineralizing agents using the pH cycling model described earlier. After remineralization, lesion depth was re-evaluated.

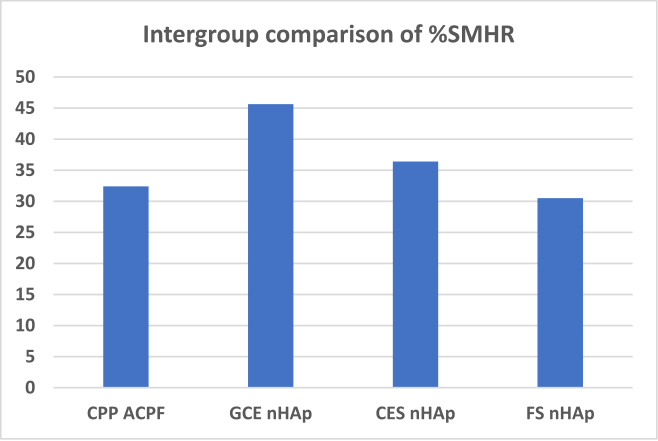

Fig. 3. Graph depicting Intergroup comparison of % Surface Microhardness Recovery.

Table 2
Intergroup comparison of Mean Lesion Depth (μm).

Group	Mean ± SD	95% CI for Mean	
		Lower	Upper
Demineralized Control	95.28 ± 8.61	89.81	100.75
CPP-ACPF	70.10 ± 5.96[a]	66.31	73.89
GCE nHAp	32.34 ± 4.79	29.29	35.38
CES nHAp	52.10 ± 7.39	47.41	56.79
FS nHAp	62.54 ± 9.06[a]	56.79	68.30

One-way ANOVA; Post hoc Tukey test.
Same superscript in lowercase indicates non-significant difference among the groups with $p \leq 0.05$ considered as statistically significant.

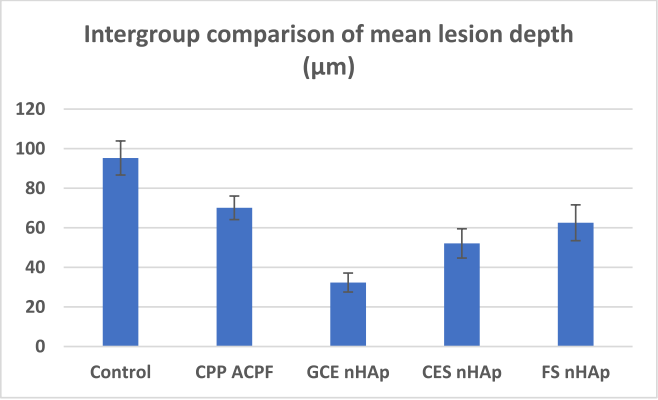

Fig. 4. Graph depicting Intergroup comparison of Mean Lesion Depth (μm).

2.12. SEM analysis

Ten samples were used for SEM analysis. Demineralized was done as previously described. Two samples (demineralized control) were then prepared for SEM by undergoing ultrasonication for 10 min, rinsed with deionized water, air-dried, gold-sputtered, and examined under a scanning electron microscope (SEM) (Nova Nano SEM™, Thermo Fisher, USA) at 5000x magnification to observe enamel surface microstructure.[28,29] After remineralization using the pH cycling model, the remaining eight samples were similarly evaluated under SEM.

2.13. EDX analysis

Thirty samples were used for EDX analysis out of which 5 served as

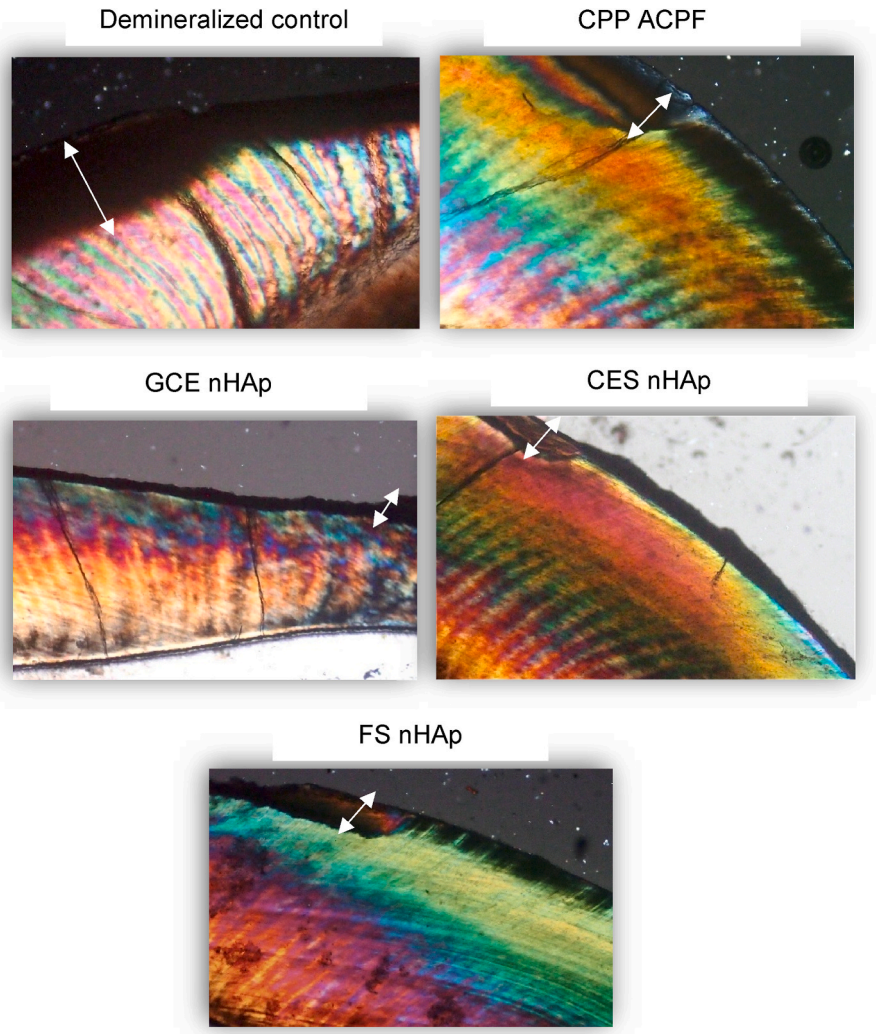

Fig. 5. Polarized Light Microscopy images of samples at 40x.

Table 3
Intergroup comparison of Ca/P Ratio.

Group	Mean ± SD	95% CI for Mean	
		Lower	Upper
Intact Control	2.20 ± 0.02	2.18	2.23
Demineralized Control	1.41 ± 0.09	1.30	1.52
CPP ACPF	1.61 ± 0.08[a]	1.51	1.71
GCE nHAp	2.01 ± 0.14	1.84	2.19
CES nHAp	1.79 ± 0.04	1.75	1.84
FS nHAp	1.60 ± 0.04[a]	1.55	1.65

One-way ANOVA; Post hoc Tukey test.
Same superscript in lowercase indicates non-significant difference among the groups with $p \leq 0.05$ considered as statistically significant.

intact controls. The remaining 25 samples underwent demineralization. After this, 5 samples (demineralized control) were first subjected to EDX analysis (Bruker, UK) in order to assess the Calcium:Phospahte (Ca/P) ratio of enamel. Following this, the remineralization of 20 samples with 5 in each reminerlization group was done by the procedure as described earlier. Finally, EDX analysis of these remineralized samples was done.

All outcome assessments, including surface microhardness testing, lesion depth evaluation, SEM analysis, and EDX analysis, were performed by a singleexaminer who was blinded to the group allocation to minimize observational bias.

2.14. Data analysis

All the collected data was entered in MS Excel. Descriptive statistics was performed by calculating mean and standard deviation for the continuous variables. The data was analyzed using IBM SPSS (Statistical Package for Social Sciences) 25.0 Version (IBM Corp 2013, New York, USA). Pairedt-testwasconductedforcomparisonofthehardness after demineralization and after remineralization. Theintergroupcomparisonofthe % SMHR wasconductedusingOne-way ANOVA test (level of significance<0.05). Pairwise comparison of %SMHR was done by Post hoc Tukey test (level of significance<0.05). Intergroupcomparisonofmean lesion depth was done by One-way ANOVA test (level of significance<0.05). Pairwise comparison of mean lesion depth was done usingPosthocTukeytest(levelofsignificance<0.05). Pairedt-testwasconductedforcomparisonofbaseline, remineralization and demineralization Ca/P ratio (level of significance<0.05). IntergroupcomparisonofCa/P was done by One-way ANOVA test (level of significance<0.05).

3. Results

For GCE nHAp, the Z-average was 49.07 nm with a PDI of 0.226, indicating moderate uniformity, while the volume-weighted size was 6.85 nm (100% by volume), meaning most particles were small and monodisperse (Fig. 2). CES nHAp showed a major particle population of

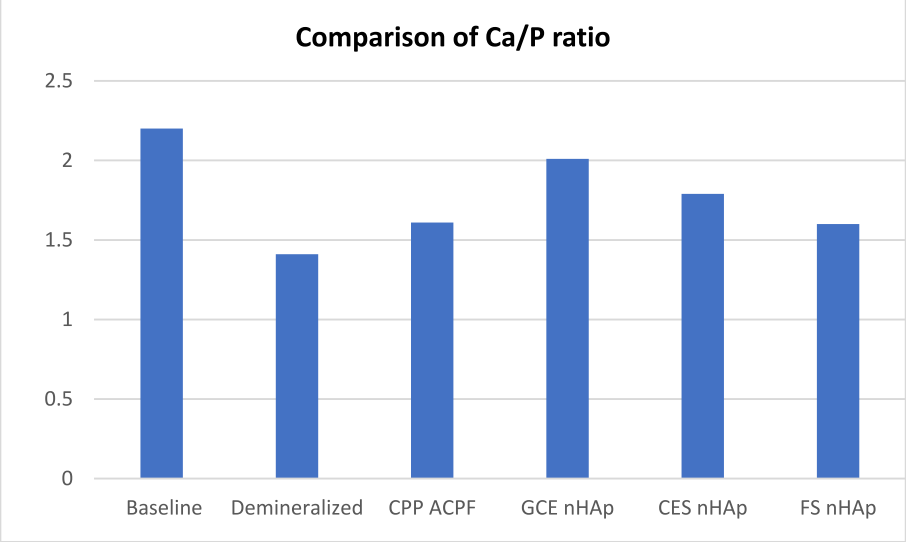

Fig. 6. Graph depicting Intergroup comparison of Ca/P Ratio.

11.2 nm (98.7% volume) and a minor population of 64.3 nm (1.3% volume). The Z-average was 58.22 nm with a PDI of 0.214, indicating almost uniform distribution (Fig. 2). FS nHAp showed a bimodal distribution with a major population of 13.2 nm (94.6% volume) and a minor population of 52.9 nm (5.4% volume). The Z-average was 49.22 nm with a PDI of 0.420, indicating that the particle distribution had moderate polydispersity (Fig. 2).

The percentage of Surface Microhardness Recovery (%SMHR) was greatest in the GCE nHAp group, followed by the CES nHAp group, while the FS nHAp group exhibited the lowest %SMHR. The intergroup differences were statistically significant ($p < 0.05$), with a 95% confidence interval and a large effect size ($\eta^2 = 0.76$). Furthermore, the %SMHR in the FS nHAp group was significantly lower compared to both the GCE nHAp and CES nHAp groups ($p < 0.05$), whereas no statistically significant difference was observed between the FS nHAp and CPP-ACPF groups ($p > 0.05$) (Table 1, Fig. 3).

The demineralized Control group exhibited the maximum mean lesion depth (μm), while the GCE nHAp had the least mean lesion depth followed by CES nHAp, FS nHAp, and CPP-ACPF. Post hoc Tukey analysis indicated that there was no significant difference between FS nHAp and CPP-ACPF groups ($p > 0.05$), whereas all other intergroup comparisons were statistically significant ($p < 0.05$) with an overall large effect size ($\eta^2 = 0.90$) and 95% confidence interval. (Table 2, Figs. 4 and 5).

A significant intergroup difference in Ca/P ratio was observed ($p < 0.05$; $\eta^2 = 0.93$). All treatment groups showed higher Ca/P ratios than the demineralized control. GCE nHAp achieved values comparable to the intact control (95% CI), while CES nHAp showed intermediate improvement. CPP-ACPF and FS nHAp were statistically similar ($p > 0.05$) (Table 3, Figs. 6 and 7).

The SEM pictures revealed that after demineralization, the treated enamel surface exhibited a loss of the enamel prism core while retaining the periphery. In contrast, the remineralized groups displayed a mineral layer formed on the enamel surface, effectively restoring the enamel prism defect (Fig. 8).

4. Discussion

Early enamel caries represents the initial stage of demineralization and can be reversed or arrested through remineralization strategies. In recent years, natural plant- and animal-derived materials have gained attention as potential remineralizing agents. Galla chinensis extract (GCE), a traditional Chinese herbal medicine, and animal-derived nanohydroxyapatite (nHAp) obtained from chicken eggshells and fish scales have demonstrated promising remineralization potential. The novelty of the present study lies in the comparative evaluation of these natural biomaterials for enamel remineralization.

In this study, GCE combined with nHAp exhibited the highest remineralizing potential, reflected by the greatest percentage surface microhardness recovery (%SMHR). This may be attributed to bioactive components of GCE, such as tannins and gallic acid, which act as calcium ion carriers and promote mineral crystal reformation.[30,31] The synergistic effect of GCE and nHAp enhanced mineral deposition, resulting in a denser and more uniform mineral layer. Similar observations were reported by Huang et al.,[22] who demonstrated superior surface crystal regularity with GCE + nHAp compared to nHAp alone.

Chicken eggshell-derived nHAp (CES nHAp) showed superior remineralization compared to CPP-ACPF and fish scale-derived nHAp (FS nHAp). This may be due to its higher crystallinity, buffering capacity, and irregular rod-like morphology, which increases surface area for acid neutralization and ion deposition. These findings align with those of Mkhize et al.,[16] who reported greater remineralizing efficacy of CES nHAp compared to.

FS nHAp. The higher microhardness observed with CES nHAp may also be attributed to its hydrophilic nature, facilitating calcium and phosphate precipitation on enamel surfaces.[32,33] CPP-ACPF demonstrated moderate %SMHR owing to its ability to release calcium and phosphate ions in combination with fluoride, forming acid-resistant fluorapatite.[7] FS nHAp also exhibited microhardness recovery comparable to CPP-ACPF, likely due to its hydroxyapatite content.[16] Accordingly, the first null hypothesis was rejected.

All remineralized groups showed a significant reduction in mean lesion depth compared to the demineralized control, with the greatest reduction observed in the GCE nHAp group. This may be attributed to enhanced ion diffusion into the lesion body facilitated by GCE, promoting subsurface remineralization.[13,22] While nHAp alone primarily promotes superficial remineralization (20–40 μm), its combination with GCE resulted in improved lesion depth reduction. CPP-ACPF, CES nHAp, and FS nHAp also significantly reduced lesion depth, although to a lesser extent, possibly due to preferential surface mineral deposition limiting deeper ion penetration. The shorter application duration (7 days) may have further constrained CPP-ACPF efficacy, as supported by Thierens et al.[34]. Polarized light microscopy confirmed these findings through a shift from positive to negative birefringence, indicating lesion arrest and mineral gain. Thus, the second null hypothesis was rejected.

SEM analysis revealed the formation of a newly developed apatite

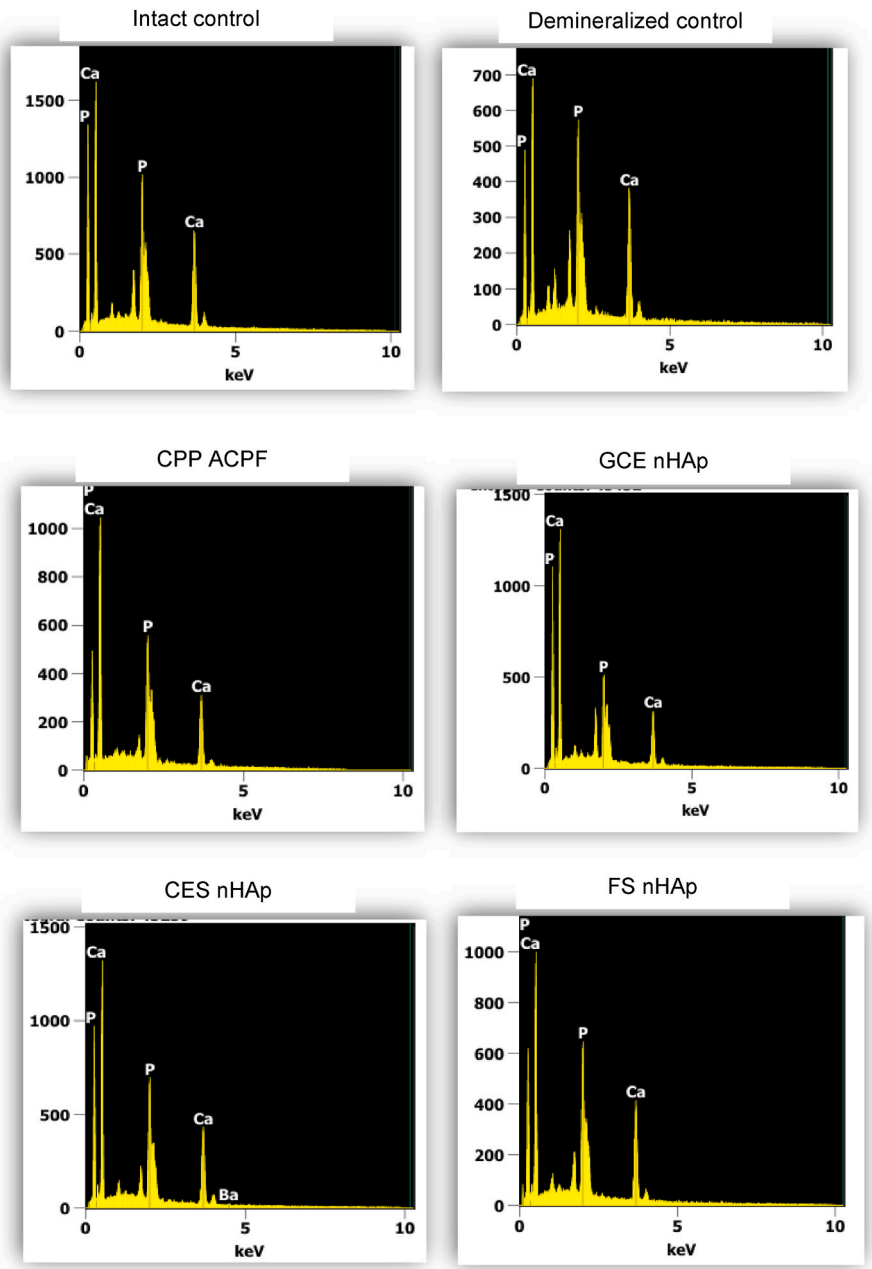

Fig. 7. EDX images of samples.

layer on the enamel surface in allremineralized groups, restoring prismatic and interprismatic structures. EDX analysis corroborated these findings, demonstrating the highest Ca/P ratio in the GCE nHAp group, followed by CES nHAp, with the lowest values observed in the CPP-ACPF and FS nHAp groups. Consequently, the third null hypothesis was rejected.

The strength of this study lies in its comprehensive evaluation of various remineralizing agents which addresses a gap in remineralization research, particularly regarding sustainable and cost-effective alternatives to fluoride-based therapies. Furthermore, the use of multiple analysis methods, such as Vickers microhardness testing, PLM, and EDX allow for a detailed understanding of how each treatment interacts with enamel at both the structural and elemental levels. The incorporation of real-world conditions, such as using human maxillary premolars and simulation of the oral environment, enhances the study's relevance and applicability. Inevitably, certain in-vitro limitations exist, such as the absence of human saliva, plaque, and varying susceptibility of teeth. Moreover, the duration of the remineralization protocol was limited to seven days, which may not adequately reflect the long-term stability, durability, and resistance of the newly formed mineral layer under prolonged acidic challenges encountered in the oral environment.Thus, future in-vivo studies are needed to validate these results over longer periods.

5. Conclusions

Within the limitations of this study, all the tested agents demonstrated a remineralizing potential on early enamel caries. Galla chinensis extract combined with nanohydroxyapatite showed the highest remineralization potential across microhardness recovery, lesion depth reduction, and Ca/P ratio enhancement, outperforming other alternatives as well as the conventional CPP-ACPF. Chicken eggshell-derived nanohydroxyapatite showed superior outcomes compared to CPP-ACPF, whereas fish scale-derived nanohydroxyapatite exhibited a

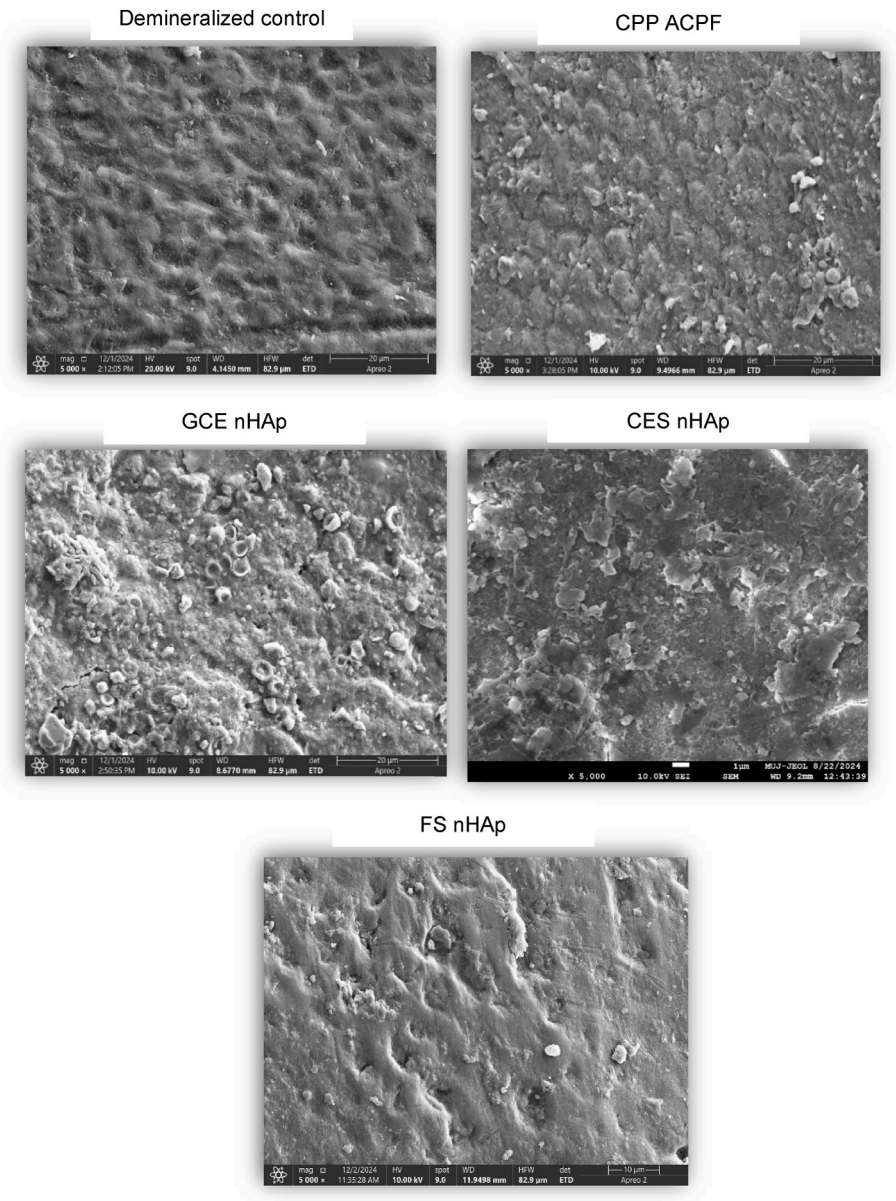

Fig. 8. SEM images of samples at 5000x.

remineralization effect comparable to CPP-ACPF. These findings suggest that natural biomimetic materials, particularly plant-based combinations, represent promising adjuncts for enamel caries management.

Patient's/Guardian's consent

Not applicable.

Ethical clearance

The protocol was reviewed and approved by the institutional ethical clearance committee underprotocol number ITSCDSR/IIEC/LD/CONS/2022-25/002.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. Machiulskiene V, Campus G, Carvalho JC, et al. Terminology of dental caries and dental caries management: consensus report of a workshop organized by ORCA and cariology research group of IADR. *Caries Res*. 2020;54(1):7–14.

2. Marsh PD, Nyvad B. The oral microflora and biofilms on teeth. In: Fejerskov O, Kidd EAM, eds. *Dental Caries. the Disease and its Clinical Management*. Oxford: Blackwell Publishing; 2003:29–48.
3. Larsen MJ. Dissolution of enamel. *Eur J Oral Sci*. 1973 Dec;81(7):518–522.
4. Sato Y, Sato T, Niwa M, Aoki H. Precipitation of octacalcium phosphates on artificial enamel in artificial saliva. *J Mater Sci Mater Med*. 2006 Nov;17(11):1173–1177.
5. Gonçalves FMC, Delbem ACB, Gomes LF, et al. Effect of fluoride, casein phosphopeptide-amorphous calcium phosphate and sodium trimetaphosphate combination treatment on the remineralization of caries lesions: an in vitro study. *Archives of Oral Biology [Internet]*. 2021 Feb 1;122 [cited 2021 Nov 14].
6. Featherstone JDB. Prevention and reversal of dental caries: role of low level fluoride. *Community Dent Oral Epidemiol*. 1999 Feb;27(1):31–40.
7. Reynolds E. Calcium phosphate-based remineralization systems: scientific evidence? *Aust Dent J*. 2008 Sep;53(3):268–273.
8. Elbahrawy E, El -Dosoky A. Remineralization potential of chicken eggshell powder in the treatment of artificially induced enamel carious lesion in permanent teeth. *Egypt Dent J*. 2019 Oct 1;65(4):3581–3593.
9. Kumar V, Itthagarun A, King N. The effect of casein phosphopeptide-amorphous calcium phosphate on remineralization of artificial caries-like lesions: an in vitro study. *Aust Dent J*. 2008 Mar;53(1):34–40.
10. Juntavee A, Juntavee N, Hirunmoon P. Remineralization potential of nanohydroxyapatite toothpaste compared with tricalcium phosphate and fluoride toothpaste on artificial carious lesionsPagano S, ed. *International Journal of Dentistry*. 2021 Mar 20;2021:1–14.
11. Tschoppe P, Zandim DL, Martus P, Kielbassa AM. Enamel and dentine remineralization by nano-hydroxyapatite toothpastes. *J Dent*. 2011 Jun;39(6):430–437.
12. Zou L, Zhang L, Li J, et al. Effect of Galla chinensis extract and chemical fractions on demineralization of bovine enamel in vitro. *J Dent*. 2008 Dec;36(12):999–1004.
13. Cheng L, Li J, Hao Y, Zhou X. Effect of compounds of Galla chinensis on remineralization of enamel surface in vitro. *Arch Oral Biol*. 2010 Jun;55(6):435–440.
14. Siva Rama Krishna D, Siddharthan A, Seshadri SK, Sampath Kumar TS. A novel route for synthesis of nanocrystalline hydroxyapatite from eggshell waste. *J Mater Sci Mater Med*. 2007 May 5;18(9):1735–1743.
15. Sampath V, Kunam D, Manimaran S, Sekar M. Evaluation of dentinal tubule occlusion and depth of penetration of nano-hydroxyapatite derived from chicken eggshell powder with and without addition of sodium fluoride: an in vitro study. *J Conserv Dent*. 2016;19(3):239.
16. Cromwell Mkhize Sandile, Onwubu Stanley Chibuzor, Mokhothu Thabang Hendrica, Selby Mdluli Phumlane, Mishra Ajay Kumar. Comparative assessment of the remineralization characteristics of nano-hydroxyapatite extracted from fish scales and eggshells. *J Appl Biomater Funct Mater*. 2023 Jan 1:21.
17. Olssom S, Berglund A, Bergman M. Release of elements due to electrochemical corrosion of dental amalgam. *J Dent Res*. 1994;73:33–43.
18. Mueller HJ. In vitro tarnish and corrosion of a consolidated silver material for direct filling applications. *Dent Mater*. 2001;17:60–70.
19. Mukherjee K, Ruan Q, Moradian-Oldak J. Peptide-mediated biomimetic regrowth of human enamel in situ. In: Papagerakis P, ed. *Odontogenesis. Methods in Molecular Biology*. 1922. New York, NY: Humana Press; 2019.
20. Sahiti JS, Krishna NV, Prasad SD, Kumar CS, Kumar SS, Babu KSC. Comparative evaluation of enamel microhardness after using two different remineralizing agents on artificially demineralized human enamel: an *in vitro* study. *J Clin Transl Res*. 2020 Sep 5;6(3):87–91.
21. Vicente A, Ortiz-Ruiz AJ, González-Paz BM, et al. Effectiveness of a toothpaste and a serum containing calcium silicate on protecting the enamel after interproximal reduction against demineralization. *Sci Rep*. 2021;11:834.
22. Huang S, Gao S, Cheng L, Yu H. Combined effects of nano-hydroxyapatite and Galla chinensis on remineralisation of initial enamel lesion in vitro. *J Dent*. 2010 Oct;38(10):811–819.
23. Nuamsrinuan N, Kaewwiset W, Limsuwan P, Naemchanthara K. Hydroxyapatite synthesized from waste eggshell via ball milling. *Adv Mod Manag*. 2017 Jun;866:12–16.
24. Buzalaf MAR, Hannas AR, Magalhães AC, Rios D, Honório HM, Delbem ACB. pH-cycling models for in vitro evaluation of the efficacy of fluoridated dentifrices for caries control: strengths and limitations. *J Appl Oral Sci*. 2010 Aug;18(4):316–334.
25. Rodrigues E, Delbem ACB, Pedrini D, Cavassan L. Enamel remineralization by fluoride-releasing materials: proposal of a pH-cycling model. *Braz Dent J*. 2010;21(5):446–451.
26. Grundmann J, Golde J, Steuer S, et al. Visualization of carious lesions with polarized and depolarized light microscopy. *Biomed Opt Express*. 2024 May 1;15(5):3018.
27. Rajan R, Krishnan R, Bhaskaran B, Kumar SV. A polarized light microscopic study to comparatively evaluate four remineralizing agents on enamel viz CPP-ACPF, ReminPro, SHY-NM and colgate strong teeth. *International Journal of Clinical Pediatric Dentistry*. 2015 Apr;8(1):42–47.
28. Meng L, Shu M, Mei P, Liang Y, Xia L. Size-controllable synthesis of hydroxyapatite nanorods via fluorine modulation: applications in dental adhesives for enhanced enamel remineralization. *BMC Oral Health*. 2025 Feb 8;25(1):204.
29. Poggio C, Lombardini M, Vigorelli P, Ceci M. Analysis of dentin/enamel remineralization by a CPP-ACP paste: AFM and SEM study. *Scanning*. 2013 Nov;35(6):366–374.
30. Chu JP, Li JY, Hao YQ, Zhou XD. Effect of compounds of Galla chinensis on remineralisation of initial enamel carious lesions in vitro. *J Dent*. 2007 May;35(5):383–387.
31. Zhang X, Deng X, Wu Y. *Remineralizing Nanomaterials for Minimally Invasive Dentistry*. Springer eBooks; 2015 Jan 1:173–193.
32. Kunam D, Sampath V, Manimaran S, Sekar M. Effect of indigenously developed nano-hydroxyapatite crystals from chicken egg shell on the surface hardness of bleached human enamel: an in vitro study. *Contemporary Clinical Dentistry [Internet]*. 2019;10(3):489–493.
33. Hassan NM, Zainab Juma Jafar, Abdul MH. Nano-hydroxyapatite preparation for the remineralization of primary tooth enamel surface subjected to liquid medication: an observational study. *Health Sci Rep*. 2023 Apr 1;6(4).
34. Thierens LAM, Moerman S, Elst C van, et al. The in vitro remineralizing effect of CPP-ACP and CPP-ACPF after 6 and 12 weeks on initial caries lesion. *J Appl Oral Sci*. 2019;27.