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Remineralization potential of strontium-doped nano-hydroxyapatite versus casein phosphopeptide-amorphous calcium phosphate with fluoride: An *in vitro* study

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Abstract:
Early enamel demineralization can progress to non-cavitated carious lesions when mineral loss exceeds natural repair. Therefore, it is of interest to assess the remineralization potential of strontium-doped nano-hydroxyapatite dentifrice (SrnHAP) compared with a topical cream containing casein phosphopeptide-amorphous calcium phosphate and fluoride (CPP-ACP-F). Hence, ninety non-carious premolar enamel samples (4×4×1 mm) were demineralized over two cycles and randomly divided into three groups of 30: Group I received a conventional dentifrice (control), Group II received CPP-ACP-F cream and Group III received SrnHAP. Calcium/phosphorus ratios were measured by SEM-EDAX at baseline, after demineralization and after remineralization and compared using one-way ANOVA with post-hoc Tukey tests. Groups II and III showed significantly greater remineralization than the control, with Group III outperforming Group II ($p<0.05$). Thus, strontium-doped nano-hydroxyapatite dentifrice showed superior remineralization compared with CPP-ACP-F and may be useful for remineralizing early carious or white spot lesions.

Keywords: Calcium phosphates, caseins, fluoride treatment, phosphopeptide, tooth remineralization

Background:
Dental caries is a biofilm-mediated, sugar-driven disease in which repeated acid exposure causes net mineral loss from enamel and dentin **[1–3].** Early enamel lesions present as subsurface porosity and white-spot changes and may be arrested or reversed when the balance shifts toward remineralization **[4–10].** Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) stabilizes bioavailable calcium and phosphate and can maintain a supersaturated mineral environment at the tooth surface; addition of fluoride may further favour fluorapatite-like mineral deposition **[11].** Nano-hydroxyapatite can penetrate surface irregularities and act as both a mineral source and a template for apatite re-precipitation **[12].** Strontium can substitute for calcium in the apatite lattice and has been investigated for its potential to reduce demineralization and support mineral recovery **[10, 13].** Experimental evidence suggests that combinations of calcium, phosphate and fluoride ions can enhance remineralization of early enamel lesions **[14].** Therefore, it is of interest to compare the remineralization potential of SrnHAP dentifrice, CPP-ACP-F topical cream and a conventional dentifrice under standardized *in vitro* conditions.

Materials and Methods:

The study received approval from the Institutional Human Ethical Committee of Saveetha Dental College and Hospitals,
Ethical Committee of Saveetha Dental College and Hospitals, with the reference number SDC/Ph.D/07/18/46.

Sample preparation:
Sample size calculation:
Sample size was estimated from the effect reported by Bhat *et al.* using G*Power, with an effect size of 0.804, $\alpha = 0.05$ and 95% power **[15].** Ninety non-carious premolars extracted for periodontal or orthodontic indications were screened under magnification to exclude cracks and fractures. Teeth were cleaned, stored in fresh normal saline at room temperature and disinfected with 10% formalin for 1 minute before specimen preparation. Crowns were sectioned at the cemento-enamel junction and standardized enamel blocks measuring approximately 4 × 4 × 1 mm were prepared with a diamond disc. Baseline elemental composition was assessed by scanning electron microscopy with energy-dispersive X-ray analysis (SEM-EDAX; JEOL JSM-840A, Tokyo, Japan) and the calcium and phosphorus values were recorded before demineralization.

Demineralization process:

Each specimen underwent two demineralization cycles using freshly prepared McInnes solution composed of 1 mL of 36% hydrochloric acid, 1 mL of 30% hydrogen peroxide and 0.2 mL of anaesthetic ether (5:5:1 by volume). The solution was applied to the enamel surface with a cotton applicator for 5 minutes. Specimens were then rinsed with deionized water, gently dried with absorbent paper and stored for 24 hours in artificial saliva containing Na3PO4 3.90 mM, NaCl 24.29 mM, KCl 17.98 mM, CaCl2 1.10 mM, MgCl2 0.08 mM, H2SO4 0.50 mM and NaHCO3 3.27 mM, adjusted to pH 7.2 with distilled water. The same procedure was repeated for a second cycle **[16].** After demineralization, calcium and phosphorus were reassessed by SEM-EDAX.

Application of remineralization agent:
Specimens were randomly allocated to three groups of 30: Group I, conventional dentifrice (control); Group II, topical CPP-ACP-F cream; and Group III, strontium-doped nano-hydroxyapatite (SrnHAP) dentifrice.

Preparation of strontium-doped nanohydroxyapatite paste:
The production of SrnHAP involved a co-precipitation process. A 50 mL solution was initially created by mixing calcium nitrate tetrahydrate (0.57 M) and strontium nitrate (0.18 M) in a 1:1 ratio. After stirring for 15 minutes, 50 mL of ammonium dihydrogen orthophosphate (0.45 M) aqueous solution was slowly added to the mixture. Ammonia was introduced to maintain a pH of 10, added at a temperature of 80 °C. After centrifugation at 5500 rpm for 30 minutes and rinse with distilled water, the resulting suspension underwent lyophilization to yield a fine powder containing 25 mol % SrnHAP. This powder was then utilized to prepare the paste. For the paste formulation, 100 gm of the unique paste was created. The ingredients included 25 mol% SrHAP, comprising 50% by weight (wt.) and sorbitol and glycerin as humectants at 30% by weight (wt.). Sodium lauryl sulfate was taken at 2% by weight (wt.), sodium alginate at 3% by weight (wt.) and deionized water at 15% by weight (wt.). The dry ingredients were meticulously ground using a mortar and pestle until finely powdered to attain a paste-like texture. Subsequently, they were combined with glycerin and deionized water. The prepared paste was then labelled and used in the present study **[13].** The Teeth in each group were treated daily with their respective allocated agents by using motorized toothbrush for 3 min twice daily (12 h interval), rinsed with running tap water and stored in artificial saliva for 28 consecutive days. After the remineralization cycle, the samples from the control and two experimental groups underwent a second SEM EDAX examination to evaluate the calcium-to-phosphorus ratio. Elemental analysis was performed using scanning electron microscopy (SEM) and energy-dispersing X-ray analysis (EDAX) on newly manufactured, unique strontium-doped nano-hydroxyapatite paste.

Statistical analysis:
Obtained values were analyzed using SPSS 22.0 software (SPSS Inc., Chicago, IL), with a p-value ($p \leq 0.05$). Descriptive statistics, including the mean and standard deviation, were employed to assess the characteristics of each group. The normality of the data was evaluated using the Shapiro-Wilks test. Inferential statistics such as one-way ANOVA (for between-group comparisons) and repeated measures ANOVA were utilized. Post Hoc assessment was done using Tukey's HSD.

Results:
At baseline, the mean calcium and phosphorus levels were 65.32 ± 0.51 and 21.13 ± 0.75, respectively. Following demineralization, these decreased to 55.22 ± 0.47 and 15.69 ± 0.53. Following the 28-day remineralization protocol, Group I demonstrated minimal recovery. Group II showed increases in calcium and phosphorus to 64.35 ± 0.37 and 19.87 ± 0.39, whereas Group III exhibited the highest post-treatment values of 65.48 ± 0.53 and 21.05 ± 0.76, respectively **(Table 1-3)**. **Table 1** compares the reported enamel elemental measurements across experimental stages. Mean calcium decreased from 65.32±0.51 to 55.22±0.47 and mean phosphorus from 21.13±0.75 to 15.69±0.53 following demineralization. After treatment, the conventional dentifrice group remained near the demineralized values (55.24 and 15.76); the CPP-ACP-F group increased to 64.35 and 19.87, whereas the SrnHAP group reached 65.48 and 21.05, respectively. **Table 2** reports the difference in group means after remineralization relative to the post-demineralization overall mean. Calcium and phosphorus differences were +0.02/+0.07 for control, +9.13/+4.18 for CPP-ACP-F and +10.26/+5.36 for SrnHAP. These are arithmetic differences of means and must not be interpreted as participant- or specimen-level paired changes. **Table 3** lists ratios of mean elemental measurements, which were 3.09 at baseline and 3.52 after demineralization, followed by 3.51 in controls, 3.24 with CPP-ACP-F and 3.11 with SrnHAP. The return toward baseline in the SrnHAP group should be interpreted with caution because the table does not specify whether SEM-EDAX data are atomic or weight percentages.

Table 1: Comparison of mean calcium and phosphorus content at baseline, after demineralization and after remineralization

Study stage/group	Calcium (Mean ± SD)	Phosphorus (Mean ± SD)
Baseline	65.32 ± 0.51	21.13 ± 0.75
After demineralization	55.22 ± 0.47	15.69 ± 0.53
Group I – Conventional dentifrice	55.24 ± 0.49	15.76 ± 0.49
Group II – CPP-ACP-F	64.35 ± 0.37	19.87 ± 0.39
Group III – SrnHAP	65.48 ± 0.53	21.05 ± 0.76

Table 2: Comparison of calcium and phosphorus recovery following remineralization among the three groups

Treatment group	Calcium recovery	Phosphorus recovery
Group I – Conventional dentifrice	+0.02	+0.07
Group II – CPP-ACP-F	+9.13	+4.18
Group III – SrnHAP	+10.26	+5.36

Recovery calculated as the treatment-group mean after remineralization minus the reported overall post-demineralization mean. These are descriptive differences, not specimen-level paired changes.

Table 3: Descriptive calcium-to-phosphorus ratios at different study stages

Study stage/group	Mean Ca/P ratio*
Baseline	3.09
After demineralization	3.52

Group I – Conventional dentifrice	3.51
Group II – CPP-ACP-F	3.24
Group III – SrnHAP	3.11

**Ratios calculated by dividing the reported group mean calcium value by the reported group mean phosphorus value. These are ratios of means, not mean individual specimen Ca/P ratios. Interpretation depends on whether the original SEM-EDAX results are atomic or weight percentages.*

Discussion:

Demineralization and remineralization are dynamic processes governed by the balance between mineral loss and mineral redeposition in enamel hydroxyapatite. When partially demineralized crystals remain structurally intact, favourable ionic conditions can permit mineral recovery rather than cavitation **[17, 18].** In the present study, SEM-EDAX was used to quantify calcium and phosphorus at baseline, after demineralization and after the 28-day remineralization protocol. Energy-dispersive X-ray analysis provides elemental information from defined surface regions, while SEM permits simultaneous evaluation of surface morphology **[19, 20].** The pH-cycling and remineralization approach was based on previously published laboratory methods **[21].** The conventional dentifrice showed little recovery after demineralization, whereas both CPP-ACP-F and SrnHAP increased calcium and phosphorus values, with the SrnHAP group showing the greatest recovery. These findings are consistent with earlier work reporting favourable remineralization and cytocompatibility of strontium-doped nano-hydroxyapatite formulations **[22].** Krishnan *et al.* similarly reported that strontium-doped nano-hydroxyapatite enhanced repair of demineralized enamel compared with comparator calcium-phosphate systems **[23].** CPP-ACP-based agents also have established remineralizing potential because casein phosphopeptides stabilize amorphous calcium phosphate and maintain calcium-phosphate ion activity at the enamel surface. Systematic and laboratory evidence supports the benefit of CPP-ACP and fluoride-containing formulations for white-spot and early enamel lesions, although results vary with formulation and study design **[24, 25].** The effectiveness of remineralization is also influenced by pH and by the surrounding calcium and phosphate concentrations; therefore, the traditional critical enamel pH should be regarded as a context-dependent value rather than a fixed threshold **[26, 27].** A plausible explanation for the greater mineral recovery observed with SrnHAP is substitution of strontium for calcium within apatite together with nano-hydroxyapatite-mediated nucleation. Strontium-containing systems have been reported to reduce enamel mineral loss and to promote apatite precipitation on demineralized dental tissues **[28, 29].** The present results do not imply that CPP-ACP-F lacks efficacy; rather, under this specific laboratory protocol, SrnHAP produced a greater restoration of the measured calcium/phosphorus profile. Previous studies have confirmed the remineralizing potential of CPP-ACP **[30]** and a synergistic effect when fluoride is incorporated into calcium-phosphate systems **[31].** Emerging approaches, including self-assembling peptides, may provide additional non-invasive strategies for early enamel repair **[32, 33].** Published *in vitro* and clinical investigations of strontium-doped nano-hydroxyapatite formulations support discussing enamel mineral recovery and other potential functional properties; the clinical trial and *in vitro* models are not directly interchangeable. **[34] [35]** these findings should be interpreted within the limits of an *in vitro* model. SEM-EDAX describes elemental recovery at sampled surface regions and does not by itself establish lesion-depth remineralization, long-term acid resistance, clinical caries arrest, or patient-centred benefit. Saliva, pellicle, oral biofilm, dietary exposures, brushing behaviour and repeated pH fluctuations in vivo may alter treatment performance. Future studies should combine surface elemental analysis with microhardness, transverse microradiography or micro-CT and repeated acid-challenge testing, followed by well-controlled clinical trials in early carious and orthodontic white-spot lesions.

Conclusion:

Strontium-doped nano-hydroxyapatite dentifrice produced greater recovery of enamel calcium and phosphorus than CPP-ACP with fluoride and the conventional dentifrice under the tested protocol. Thus, data support further laboratory and clinical evaluation of SrnHAP for early carious and white-spot lesions, but they do not by themselves establish clinical superiority.

Limitations:

This study used artificially demineralized enamel specimens under controlled laboratory conditions and therefore did not reproduce the effects of saliva, pellicle, biofilm, diet, patient behaviour, or repeated clinical acid challenges. Only one SrnHAP formulation and one CPP-ACP-F comparator were tested. Future studies should compare additional remineralizing agents, include complementary measures such as microhardness and lesion-depth analysis, assess resistance to subsequent demineralization and evaluate safety and effectiveness in clinical white-spot and early-caries lesions.

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Data availability: The data generated in this study are available from the corresponding author on reasonable request.

Ethics approval: The study protocol was approved by the Scientific Review Board (reference SDC/Ph.D/07/18/46).

Conflicts of interest: The authors declare no conflicts of interest.

References:

[1] Balakrishnan M *et al. Aust Dent J*. 2000 **45:**235 [PMID: 11225524]
[2] Mehta R *et al. Indian J Dent Res.* 2013 **24:**681[PMID: 24552927]
[3] Cochrane NJ *et al. J Dent Res*. 2010 **89:**1187 [PMID: 20739698]
[4] Mizrahi E. *Am J Orthod.* 1982 **82:**62 [PMID: 6984291]
[5] Shankarappa S *et al. J Orthod Sci.* 2024 **13:**21 [PMID:38784071]

[6] Mittal R *et al. Int J Clin Prev Dent*. 2017 **13**:1. [DOI: 10.15236/ijcpd.2017.13.1.1]
[7] Tahmasbi S *et al. J Dent Res Dent Clin Dent Prospects*. 2019 **13:**36 [PMID: 31217917]
[8] Kobeissi R *et al. Int J Clin Pediatr Dent.* 2020 **13:**451 [PMID: 33623327]
[9] Featherstone JDB & Chaffee BW *Adv Dent Res.* 2018 **29:**9 [PMID: 29355423]
[10] Sedlakova Kondelova *et al. Sci Rep.* 2020 **19:**20211 [PMID: 33214593]
[11] Farooq I *et al. King Saud Univ. J. Dent. Sci*. 2013 **7:**51. [PMID: 34842827]
[12] Pushpalatha C *et al. Saudi Dent J.* 2023 **35:**74 [PMID: 37817794]
[13] Jayasree R *et al. J Mater Sci Mater Med*. 2017 **28:**95. [PMID: 28502026]
[14] Dai LL *et al. Materials Basel*. 2019 **12:**4183 [PMID: 31842454]
[15] Bhat DV *et al. Int J Clin Pediatr Dent*. 2022 **15**:S221. [PMID: 35645530]
[16] https://www.semanticscholar.org/paper/Influence-of-McInnes-bleaching-agent-on-hardness-of-Journal-Hora/286f68385085ed697f8350beaca52cb373a2187c
[17] Abou Neel EA *et al. Int J Nanomedicine*. 2016 **11:**4743 [PMID: 27695330]
[18] Thierens LAM *et al. J Appl Oral Sci*. 2019 **27**:e20180589 [PMID: 31116280]
[19] Khan MSI *et al. Sci Rep*. 2020 **10:**2368. [PMID: 32047250]
[20] Scimeca M *et al. Eur J Histochem.* 2018 **62:**2841. [PMID: 29569878]
[21] Shetty S *et al. J Conserv Dent*. 2014 **17**:49 [PMID: 24554861]
[22] Rajendran R *et al. J Conserv Dent*. 2020 **23**:330. [PMID: 33623231]
[23] Krishnan V *et al. Dent Mater*. 2016 **32**:646. [PMID: 26922626]
[24] Chen H *et al. Am J Orthod Dentofacial Orthop*. 2013 **143**:376 [PMID: 23452972]
[25] Penumatsa NV *et al. J Pharm Bioallied Sci*. 2015 7: S583 [PMID: 26538923]
[26] Meyer F *et al. Open Dent J*. 2018 **12**:406 [PMID: 29988215]
[27] Muhammad SA & Ahmed BM. *Erbil Dent J*. 2022 **5**:58. [DOI:10.15218/edj.202.8]
[28] Wang YL *et al. J Formos Med Assoc*. 2019 **118**:39 [PMID: 29395390]
[29] Fredholm YC *et al. J R Soc Interface*. 2012 **9**:880 [PMID: 21993007]
[30] Balakrishnan A *et al. J Conserv Dent*. 2013 **16**:375. [PMID: 23956545]
[31] Patil *et al. Bioinformation*. 2026 **22:**1339 [DOI: 10.6026/973206300221339]
[32] Xavier GD *et al. J Conserv Dent Endod*. 2024 **27**:29. [PMID: 38389734]
[33] Babu B *et al. Cureus*. 2025 **17**:e95416. [PMID: 41306178]
[34] Rajendran R *et al. SAGE Open Med*. 2024 **12**:20503121231221634. [PMID: 38162913]
[35] Mathew R *et al. Mater Today Proc*. 2021 46:5975. [DOI: 10.1016/j.matpr.2020.12.402]

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