

Introduction:

- Fluoride is a well-established anti-cariogenic agent. Recently, casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) has gained attention for caries management.
- ACP is a supersaturated Ca–P reservoir. CPP binds to the tooth pellicle from one side and binds ACP from the other side, so localize ACP at the tooth surface. CPP binds to the pellicle by occupying protein sites necessary for the adhesion of *S. mutans*, helping to reduce *S. mutans* as well. Another role of CPP is to stabilize ACP, preventing it from crystallizing and keeping it in an amorphous, ready-to-precipitate state.
- In CPP-ACFP, fluoride is incorporated into the CPP-ACP, promoting fluorapatite formation.
- With increasing use of low- or no-fluoride agents, comparing their remineralization efficacy has become essential (1,2).



Materials and Methods:

Step 1: Specimen Preparation

Twenty-five extracted or exfoliated anterior and posterior primary teeth were collected. The roots were removed, and each crown was sectioned mesiodistally into two halves. Specimens were mounted in epoxy resin, leaving the buccal or lingual enamel surface exposed. The enamel surfaces were polished to obtain a smooth surface. Two specimens were obtained from each tooth.



Step 2: Pre-demineralization Microhardness Measurement

The specimens' Vickers Hardness Number (VHN) was calculated using a digital microhardness tester (Koop, model MH3, Iran).



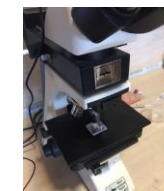
Step 3: Demineralization Process

Specimens were immersed in a demineralizing solution at 37°C for 3.5 hours.



Step 4: Post-demineralization (Baseline) Microhardness Measurement

The specimens were rinsed and stored in artificial saliva. Surface microhardness (baseline) was re-measured.



Step 5: Grouping and Treatment Protocol

Specimens divided into five groups:

- Group 1: Distilled water (control)
- Group 2: CPP-ACP paste
- Group 3: CPP-ACFP paste (900 ppm F)
- Group 4: Fluoridated toothpaste (1450 ppm F)
- Group 5: 0.4% stannous fluoride gel (1000 ppm F)

For Groups 2, 3, and 4, materials were prepared as a slurry (1:3 ratio of material to artificial saliva). These were applied daily (*morning and evening*) using a swab for 3 minutes, followed by a 3-minute undisturbed occluded period. Specimens were rinsed with distilled water for 1 second after each application. For Group 5, stannous fluoride gel was applied according to manufacturer instructions and left undisturbed for 30 minutes before rinsing with distilled water.



pH Cycling Regimen

After morning treatment, specimens underwent daily pH cycling. Immersion in demineralizing solution for 30 minutes → Rinsing with distilled water → Immersion in remineralizing solution for 2.5 hours → Storage in artificial saliva for 10 minutes prior to evening treatment. Specimens were stored overnight in artificial saliva. Solutions were refreshed daily.



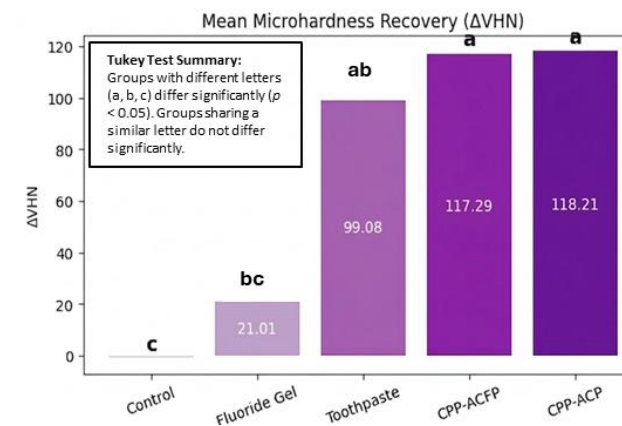
Step 6: Final Microhardness Measurement

After 20 days of treatment, surface microhardness was measured again using a digital microhardness tester to obtain the final Vickers Hardness VHN values.



Results:

- Based on one-way ANOVA, no significant differences in baseline (after demineralization) microhardness were observed among the five groups ($P = 0.310$), confirming initial comparability.
- The greatest increase in microhardness (ΔVHN) was observed in the CPP-ACP group, followed closely by the CPP-ACFP group, fluoridated toothpaste, and fluoride gel.
- Tukey analysis revealed no significant difference among CPP-ACP, CPP-ACFP, and fluoridated toothpaste. However, CPP-ACP and CPP-ACFP showed significantly greater remineralization than fluoride gel and control. The difference between fluoridated toothpaste and fluoride gel was not statistically significant. Fluoridated toothpaste was superior to the control, whereas fluoride gel was not.



Comparison (Microhardness)	p-value
CPP-ACP > CPP-ACFP	0.998
CPP-ACP > Fluoridated Toothpaste	0.609
CPP-ACFP > Fluoridated Toothpaste	0.795
CPP-ACP > Fluoride Gel	0.005
CPP-ACFP > Fluoride Gel	0.013
CPP-ACP > Control	0.001 >
CPP-ACFP > Control	0.001
Fluoridated Toothpaste > Fluoride Gel	0.182
Fluoridated Toothpaste > Control	0.022
Fluoride Gel > Control	0.887

Discussion:

- CPP-based agents demonstrated greater remineralization than fluoride-containing agents, with CPP-ACP showing slightly higher effectiveness than CPP-ACFP.
- The lower efficacy of fluoride agents may be due to the formation of a superficial fluoride-rich layer that limits deeper ion diffusion. These findings align with previous studies and suggest that CPP-ACP products can serve as effective alternatives or adjuncts to fluoride therapy, particularly for individuals at risk of excessive fluoride exposure (3,4).

References:

- Baccolini V, da Silva LP, Teixeira L, et al. The role of casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) in white spot lesion remineralization: a systematic review. *J Funct Biomater.* 2025;16(8):272.
- Pukallus ML, Plonka KA, Holcombe TF, et al. A randomized controlled trial of a 10% CPP-ACP cream to reduce mutans streptococci colonization. *Pediatr Dent.* 2013;35:550-555.
- Zhou C, Zhang D, Bai Y, Li S. Casein phosphopeptide–amorphous calcium phosphate remineralization of primary teeth early enamel lesions. *J Dent.* 2014;42(1):21-29.
- Oliveira GM, Ritter AV, Heymann HO, et al. Remineralization effect of CPP-ACP and fluoride for white spot lesions in vitro. *J Dent.* 2014;42(12):1592-1602.