

# The Role of Fluoride and Casein Phosphopeptide – Amorphous Calcium Phosphate in Remineralization

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## Aim

The purpose of the studies was to evaluate the effect of CPP-ACP on teeth and the level of remineralization with fluoride and CPP-ACP or both together.

## Introduction

The prevalence of dental caries is increase due to the dietary change and the conservative treatment of dental caries is to remove the affected enamel and dentin, then replace it with restoration material. Later on, scientists use fluoride as a preventive agent into tooth to enhance the anti-caries activity. Fluoride is one of the common elements, which has strongly cariostatic. It involves in two processes, demineralization and remineralization, which control the progression and reversal of carious lesions in teeth. Treatment with 5000ppm F significantly enhanced remineralization and inhibited demineralization when compared with treatments with 1500 ppm <sup>[2]</sup>.

In recent studies, a kind of novel preventive agents, casein phosphopeptide - amorphous calcium phosphate (CPP-ACP) develops, which slows the progression of caries and remineralizes enamel subsurface lesions. Longbottom C. et al. (2009), an ideal caries preventive material should release calcium and phosphate in oral cavity <sup>[1]</sup>. Thus, manufactures produce MI paste, which is the first product, contains RECALDENT™ (CPP-ACP).

Casein phosphopeptides – amorphous calcium phosphate (CPP-ACP) are peptides that are derived from milk sticky protein complex with calcium and phosphate. CPP-ACP has been demonstrated to increase the levels of calcium and phosphate ions significantly in supra-gingival plaque and also the remineralization of enamel subsurface lesion *in situ* <sup>[3]</sup>. Casein phosphopeptide can also decrease the count of *Streptococcus Mutans* as it has the ability to integrate in the pellicle <sup>[4]</sup>.

Moreover, the synergistic effect of CPP-ACP and fluoride in reducing caries may be attributable to the formation of CPP-ACP <sup>[3]</sup>; due to the increased incorporation of fluoride ions on plaque, will increase concentrations of calcium and phosphate ions in oral environment.

## Method and Result

### FE-SEM observation of demineralization on treated tooth surface

The specimens were prepared into the sliced bovine teeth with both enamel and dentine. One group of specimens was treated with 0.1 M lactic-acid buffer solution then stored in artificial saliva (pH 7.0). The additional two groups of specimens were put in a 10-fold diluted solution of CPP-ACP or placebo paste without CPP-ACP for 10 min before storage in a demineralizing solution. They were exposed to the demineralizing solution twice a day throughout the 4-week test period. Observations were carried out daily for the 7, 14, 21 and 28 days after the test period.

The result of demineralization of the enamel surfaces was more obvious in the demineralization (DE) and negative control (PP) groups. However, enamel specimens which treated with CPP-ACP paste (TM groups) revealed slight changes in the morphology. (Fig.1) On the other hand, the demineralization of the dentin surfaces was more pronounced over the test period in the DE and PP groups. By contrast, the dentin specimens in the TM group showed relatively minor changes in their morphological features. (Fig. 2) <sup>[5]</sup>

### Plaque fluoride level study

The study includes 3 different type of mouthrinses: (1) 2% w/v CPP-ACP (RECALDENT™) with 450 ppm F as NaF in deionized water; (2) 450 ppm F as NaF in deionized water; (3) a placebo control rinse as deionized water. The rinses were adjusted to pH 7.0. The participants used 15 mL of each rinse for 60 sec. 3 times a day for 4 days. Participants refrained from all oral hygiene procedures and each of them crossed over to use each mouth rinse, with a four-week washout period between treatments.

The result found out that both fluoride-contained rinses produced an increase in plaque fluoride levels. The plaque fluoride level of 450 ppm F rinse was nearly double than the placebo control group. The addition of 2 % CPP-ACP plus 450 ppm F significantly increased the fluoride ions into plaque, which was over double than the fluoride-alone rinse. <sup>[6]</sup>

### The evaluation of lesion depth on remineralized enamel

Thirty extracted human premolars without any enamel lesions. One group were placed in a demineralizing solution with pH 4.46 for 8 hours and removed in artificial saliva for 1 hour, finally placed in a remineralizing solution (pH 7.0) for 24 hours. This cycling continued for 14 days. And the second group was applied fluoridated tooth paste and allowed the teeth sit for 5 min; the further treatment same as group 1. The third group was applied CPP-ACP containing past (MI paste RECALDENT™) 5 min and were further treated similar to group 1.

The result of the mean lesion depth observed in the control group was 55.47 μm. The mean lesion depth observed in the fluoridated tooth paste-treated group was 23.88 μm. The mean lesion depth observed in the CPP-ACP tooth paste-treated group was 19.05 μm. (Table. 1) <sup>[7]</sup>

### Microradiography analysis of the effect of CPP-ACP with fluoride *in vitro* study

The permanent teeth were placed in demineralizing solution for 96 hours to produce artificial caries-like lesions. The teeth were suctioned into 100–150 μm thick samples and randomly assigned to four groups: Group A, fluoride toothpaste 1100 ppm was used as a positive control and Group B, non-fluoride toothpaste; Group C, topical coating of CPP-ACP; and Group D as a topical coating after treating with the same fluoridated as in Group A.

The result of the lesion depth decreased significantly by 7 percent in Group A (Fig. 3), 10.1 percent in Group C (Fig. 5) and 13.1 percent in Group D (Fig. 6) (Paired t-test, p < 0.05), while in Group B the lesion depth increased significantly by 23 percent. (Fig. 4) <sup>[8]</sup>

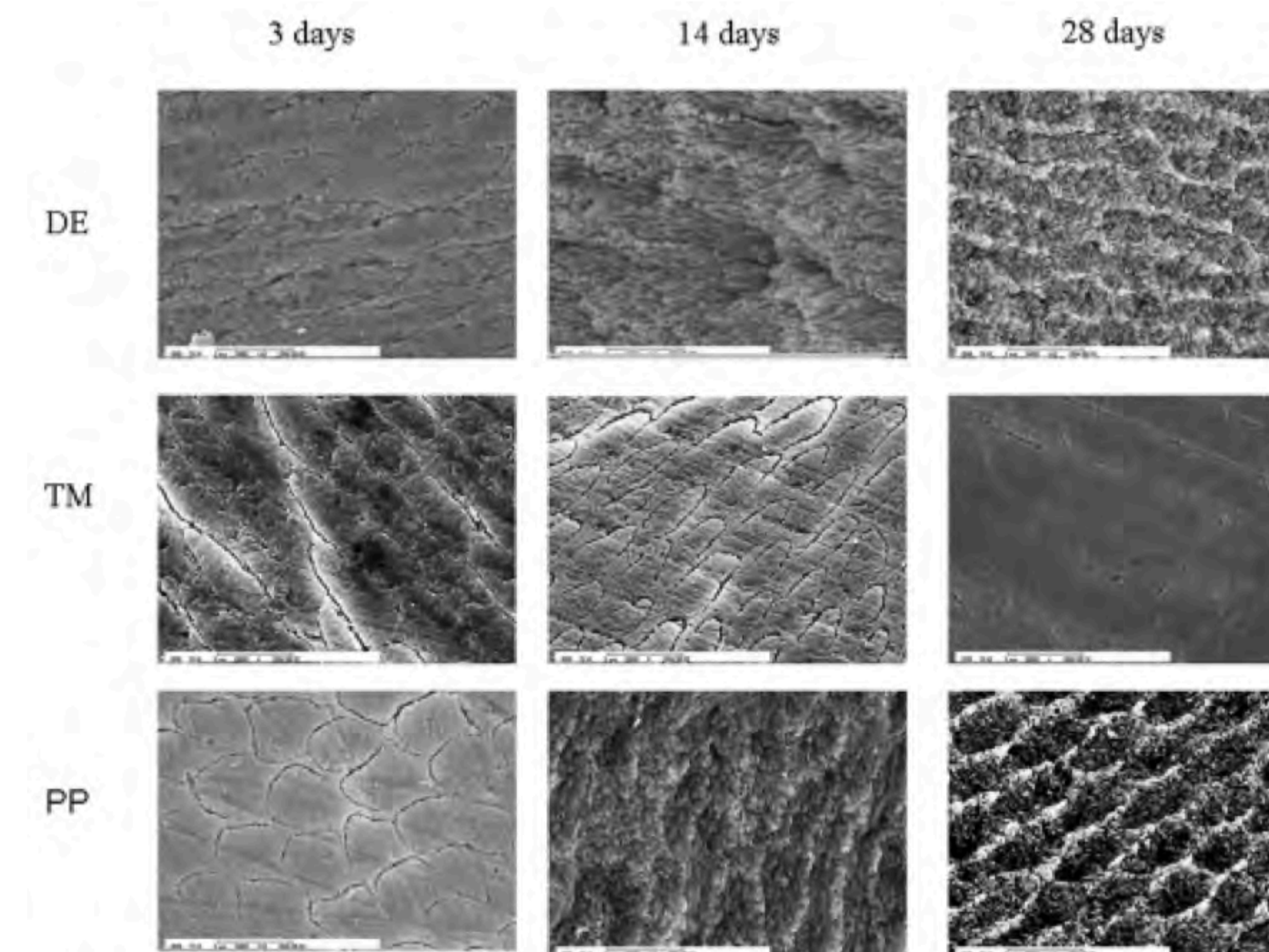


Fig. 1 FE-SEM observation of enamel surfaces (original magnification: x5000)

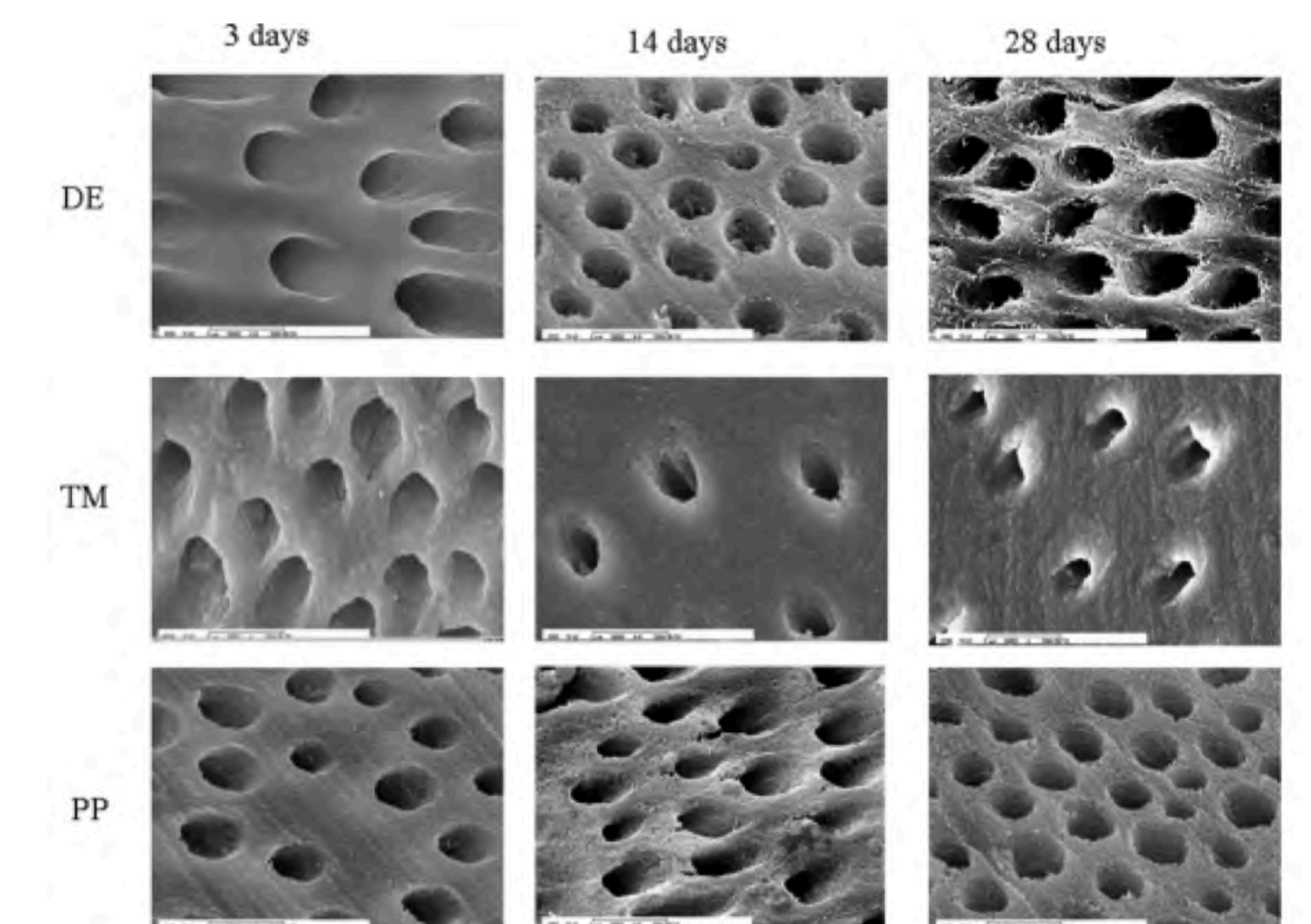


Fig. 2 FE-SEM observation of dentin surfaces (original magnification: x5000)

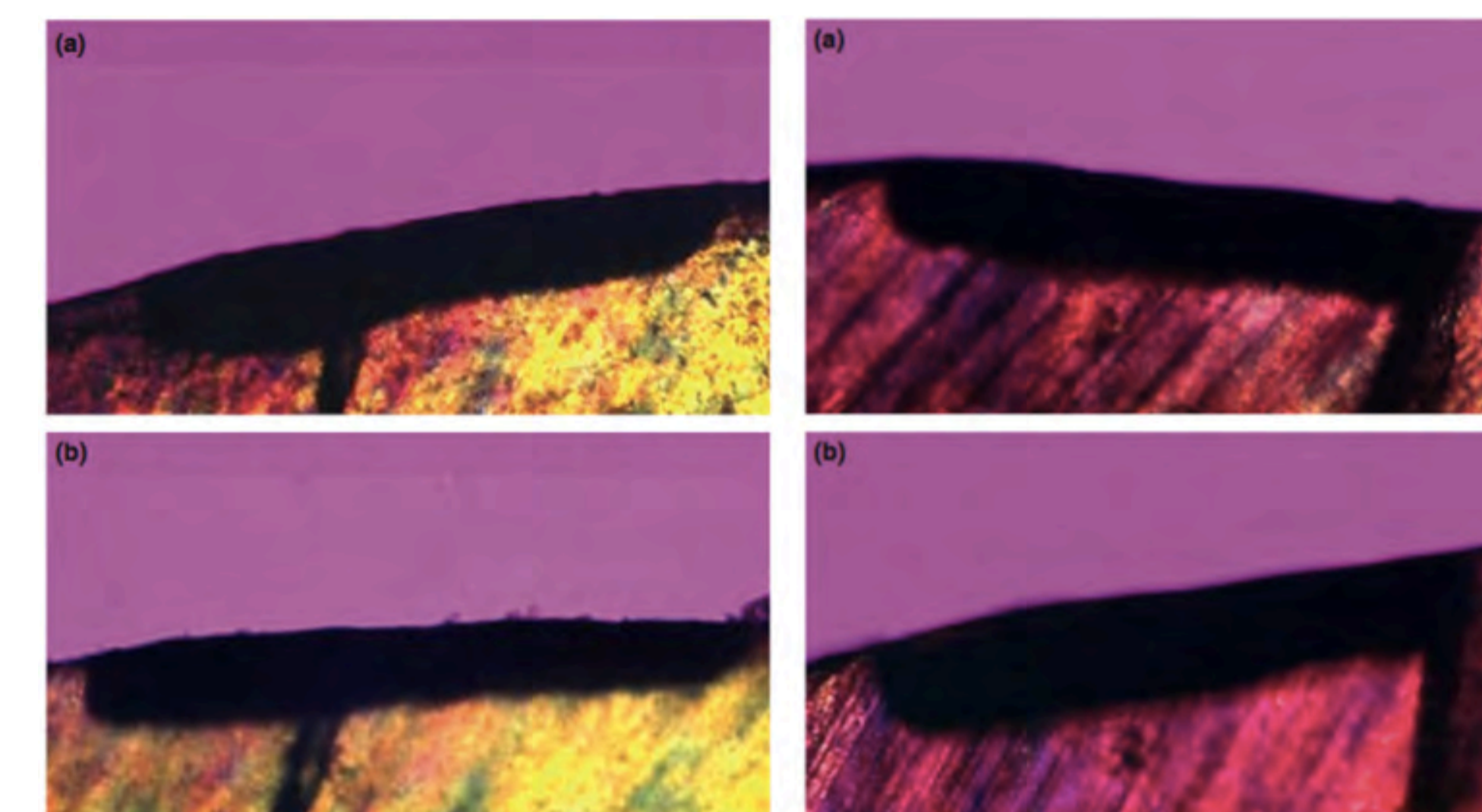


Fig. 3 Polarized light photomicrograph of an enamel lesion in Group A (a) before and (b) after treatment

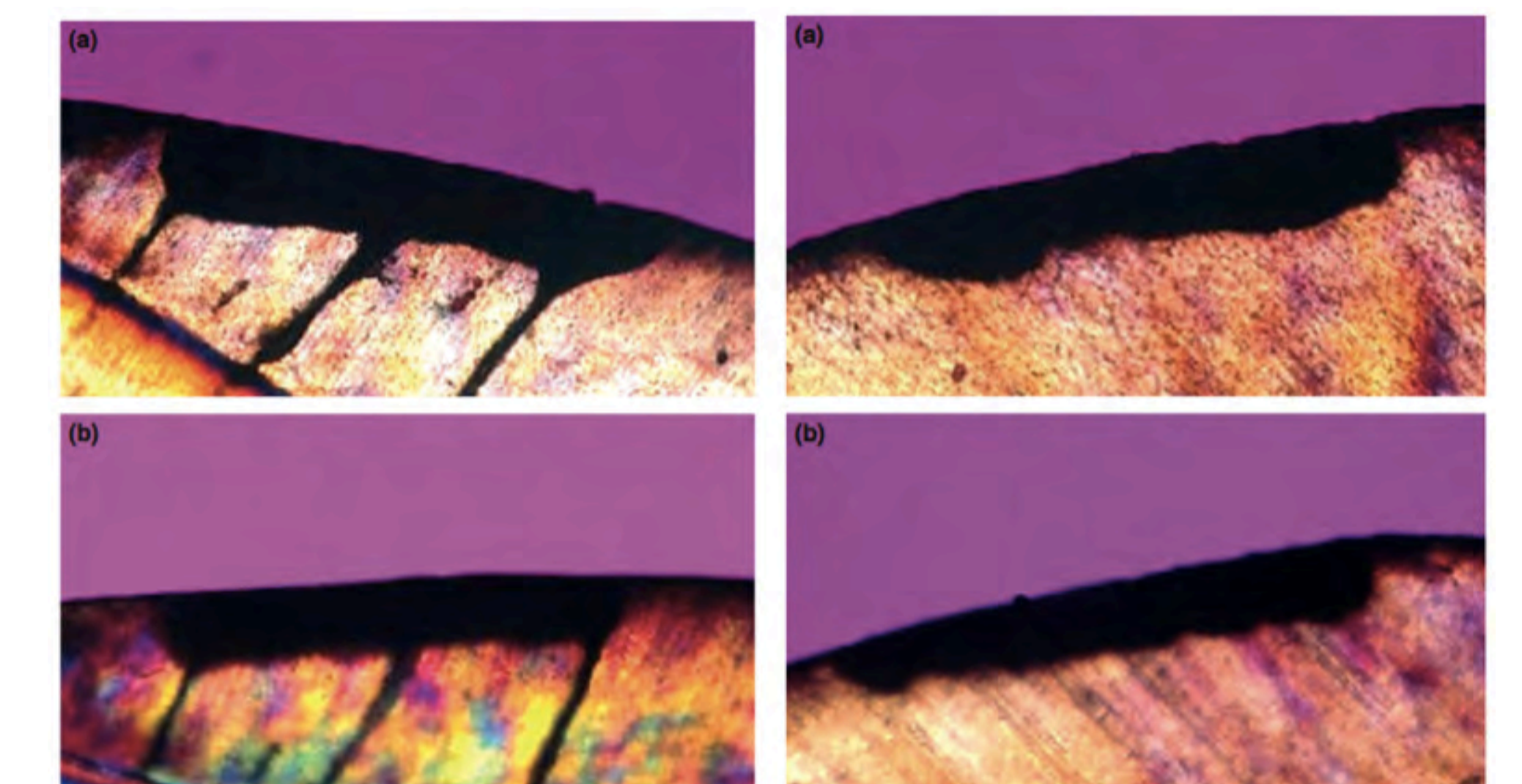


Fig. 4 Polarized light photomicrograph of an enamel lesion in Group B (a) before and (b) after treatment

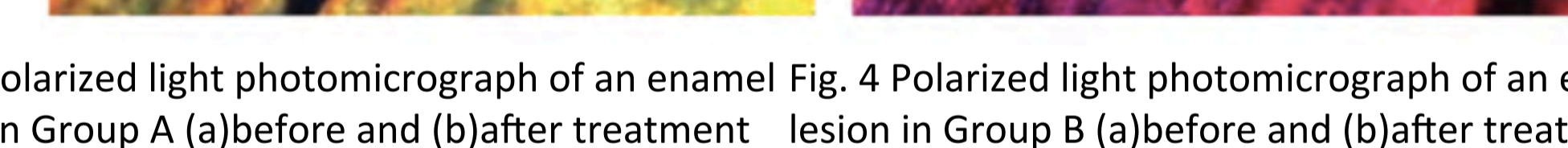


Fig. 5 Polarized light photomicrograph of an enamel lesion in Group C (a) before and (b) after treatment



Fig. 6 Polarized light photomicrograph of an enamel lesion in Group D (a) before and (b) after treatment

Fig. 3 Polarized light photomicrograph of an enamel lesion in Group A (a) before and (b) after treatment

Fig. 4 Polarized light photomicrograph of an enamel lesion in Group B (a) before and (b) after treatment

Fig. 5 Polarized light photomicrograph of an enamel lesion in Group C (a) before and (b) after treatment

Fig. 6 Polarized light photomicrograph of an enamel lesion in Group D (a) before and (b) after treatment

## Discussion and Conclusion

Based on the results, CPP-ACP has an effective reducing demineralization of enamel and dentin more than the placebo paste <sup>[5]</sup>. And compared the lesion depths followed by enamel surfaces treated with fluoride paste, CPP-ACP exhibited the least lesion depths <sup>[7]</sup>. Based on E.C. Reynolds et al. (2008) the effect of fluoride

paste is double than non-fluoride paste and the effect of CPP-ACP is more double than fluoride <sup>[6]</sup>. Moreover, we obtain the more inhibited demineralization and increased remineralization in the synergy of CPP-ACP and fluoride <sup>[8]</sup>. Lots of similar studies show the same outcome. CPP not only increase fluoride incorporation into plaque but also increased into subsurface enamel, compared with fluoride alone. The 2% CPP-ACP dentifrice was superior to which contain 1100 ppm-F and achieves a similar level of remineralization with the 2800 ppm-F <sup>[6]</sup>. These results highlight the importance of the present of calcium and phosphate ions for net remineralization while topical fluoride are applied; because CPP-ACP is a nano-complex, it increases concentration of calcium and phosphate on the tooth surface which would drive diffusion into enamel, producing higher activities of ions in the subsurface lesion, resulting in higher levels of remineralization and fluoride incorporation into the mineral phase.

## Reference

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Table 1. Mean lesion depth (μm) for the three groups

Type of taste	N	Min	Max	Mean	Std.	Skewness		Kurtosis		
		Sat.	Sat.	Sat.	Sat.	Std. err.	Std.	Std. err.		
Control	10	51.29083	64.97137	55.47555	4.13173	1500	0.687	2.400	1.334	
Fluoride	Mean Lesion Depth (um)	10	19.32812	29.81687	23.87731	3.01625	0.543	0.687	0.581	1.334
	Valid N (List wise)	10								
CPP-ACP	Mean Lesion Depth (um)	10	16.55643	22.71868	19.05012	1.79365	0.627	0.687	0.966	1.334
	Valid N (List wise)	10								