Original Article

The longevity of casein phosphopeptide–amorphous calcium phosphate fluoride varnish's preventative effects: *Assessment of white spot lesion formation*

Moufida Abufarwa[a]; Amal Noureldin[b]; Phillip M. Campbell[c]; Peter H. Buschang[d]

ABSTRACT

Objectives: To test how long casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) fluoride varnish prevents enamel demineralization in vitro.

Materials and Methods: Human molars and premolars were sectioned buccolingually and randomly assigned to two groups. Standardized pretreatment images of enamel surfaces were obtained using FluoreCam. The control group received no treatment, and the experimental group received an application of CPP-ACP fluoride varnish. Over simulated periods of 2, 4, 8, and 12 weeks, specimens were placed in a toothbrushing simulator, thermocycled, subjected to 9 days of pH cycling, and imaged with FluoreCam. Samples were sectioned and polished for polarized light microscope (PLM) evaluation.

Results: There were statistically significant time ($P < .001$) and varnish ($P < .001$) effects on area, intensity, and impact of enamel demineralization. The control group showed significant and progressive demineralization over the 12 weeks ($P < .001$). The experimental group revealed no significant demineralization during the first 4 weeks ($P > .05$) and significant ($P < .001$) increases thereafter. Experimental demineralization after 12 weeks was comparable to 2-week demineralization in the controls, with significant between-group differences ($P < .001$) in enamel demineralization at all time points. PLM of the control and experimental groups revealed lesion depths of 90 $\pm$ 34 μm and 37 $\pm$ 9 μm, respectively.

Conclusions: Within the limitations of this in vitro study, CPP-ACP fluoride varnish prevents enamel demineralization for at least 4 weeks and limits demineralization up to 12 weeks. (*Angle Orthod.* 2019;89:10–15.)

KEY WORDS: Casein phosphopeptide–amorphous calcium phosphate; Fluoride varnish; FluoreCam; White spot lesions; Enamel demineralization; Light-induced fluorescence

INTRODUCTION

White spot lesions (WSLs) are clinical signs of enamel demineralization that, if not treated at an early stage, may progress to dental caries or arrest, leaving a permanent white scar.[1] The risk of WSLs increases with fixed orthodontic appliances, especially in patients with poor oral hygiene. WSLs most often occur on the buccal surfaces of the maxillary lateral incisors, followed by canines, premolars, and central incisors.[2,3] Visible WSLs occur in approximately 28% for patients treated in university and private dental practice settings.[3,4] They can be seen as early as 4 weeks after orthodontic appliance placement.[5]

Fluoride varnish is one of the ways to reduce WSLs in orthodontic patients.[6] Prolonged contact time with the varnish enhances the fluoride uptake of enamel, especially in the outermost layers, and promotes the

[a] Graduate student, Department of Biomedical Sciences, Texas A&M University College of Dentistry, Dallas, Tex.

[b] Associate Professor, Department of Public Health Sciences, Texas A&M University College of Dentistry, Dallas, Tex.

[c] Professor Emeritus, Department of Orthodontics, Texas A&M University College of Dentistry, Dallas, Tex.

[d] Regents Professor and Interim Head, Department of Orthodontics, Texas A&M University College of Dentistry, Dallas, Tex.

Corresponding author: Dr Moufida Abufarwa, Texas A&M University College of Dentistry, 3302 Gaston Avenue, Dallas, TX 75246
(e-mail: mofify@gmail.com)

Accepted: July 2018. Submitted: February 2018.
Published Online: September 12, 2018

© 2019 by The EH Angle Education and Research Foundation, Inc.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-05-16 via free access

formation of calcium fluoride (CaF_2) on the tooth's surface.[7,8] CaF_2 acts as an intraoral fluoride reservoir, releasing its calcium and fluoride ions when the oral pH falls below 5 and reversing the demineralization process.[7] Fluoride retention and its preventive effect gradually decrease over time.[8]

The longevity of fluoride varnish's preventive effect has not been clearly established. According to American Dental Association (ADA) recommendations, fluoride varnish should be applied every 3 to 6 months.[9] This recommendation may be inappropriate because one fluoride varnish application at the beginning of the orthodontic treatment does not prevent WSLs after 3 months.[10] Fluoride varnish applications every 6 weeks are only 30% more successful in reducing WSLs in vivo than placebo applications.[11] The ADA recommendation was based on studies assessing advanced stages of demineralization (ie, dental caries). New technologies are now available that can detect lesions at earlier stages of WSL development.[12,13]

WSL development is a dynamic process in which enamel demineralization exceeds remineralization.[14] Manufacturers have tried to improve the efficacy of fluoride varnish by adding calcium and phosphate ions.[15] Recently, a fluoride varnish combined with casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) has been developed (MI varnish).[16] CPP-ACP is an amorphous form of calcium phosphate (ACP) stabilized by a phosphopeptide from the milk protein casein (CPP).[17] Topical application of CPP-ACP releases calcium and phosphate ions, which enhances remineralization and prevents demineralization.[9,18] The incorporation of CPP into the salivary pellicle inhibits the adhesion of cariogenic bacteria (*Streptococcus mutans*), which produce noncariogenic plaque.[19] Several studies have demonstrated the anticariogenic properties of CPP-ACP[20,21] and its synergistic effect with fluoride.[22] Fluoride varnish containing CCP-ACP is more effective in increasing the acid resistance of enamel than other varnishes[17] and releases more fluoride, calcium, and phosphate.[16]

FluoreCam (DARZA, Noblesville, Ind) is an optical device designed for early WSL detection. When the enamel is exposed to certain light wavelengths, it emits a green fluorescent light that can be detected by the FluoreCam. Demineralized enamel emits less light; the FluoreCam depicts lesions as dark gray areas. FluoreCam generates three outputs: area of enamel demineralization, intensity (light-intensity loss), and impact of demineralization (product of intensity and area). Changes over time can be used to monitor the progression or regression of lesions.[20]

The aim of the present longitudinal in vitro study was to determine how long CPP-ACP fluoride varnish prevented WSLs formation using the FluoreCam. The longevity of the preventive effect of CPP-ACP containing fluoride varnish has not been tested.

MATERIALS AND METHODS

Study Design

Enamel demineralization of the control and experimental group was monitored over 12 weeks in vitro (Figure 1). Oral conditions were simulated by thermocycling and tooth brushing. After 2, 4, 8, and 12 weeks, pH cycling was conducted, and enamel demineralization was evaluated using the FluoreCam system. Representative samples were examined at the end of the study using a polarized light microscope (PLM).

Sample Size and Power Analysis

Assuming an effect size of 0.72, which was based on published estimates,[23] a sample size of $n = 40$ per group was necessary to achieve a type I error rate of 5% and a power of 99%.[24] Forty sound human permanent molars and premolars were cut into halves so that both buccal and lingual surfaces could be evaluated.

FluoreCam Imaging

The specimens were randomly assigned to either a control or experimental group. Pretreatment images of the enamel surfaces were recorded under standardized conditions using the FluoreCam.[23] The FluoreCam device was positioned a fixed distance from a mounting table. A mold was prepared for each specimen using a vinyl polysiloxane impression material (Exaflex Putty, GC America, Inc, Alsip, Ill), into which the root of each specimen was inserted horizontally. The FluoreCam was placed touching the enamel, and the impression material was molded around the tip, producing an indentation. The indentation served to ensure the same FluoreCam position and orientation for subsequent imaging. An image of each specimen was captured, and the baseline areas (mm^2), light intensities (pixels), and impacts of demineralization ($pixel.mm^2$) were recorded. FluoreCam imaging was repeated for each specimen after 2, 4, 8, and 12 weeks.

Treatment Application

The specimens were covered with an acid-resistant nail polish (Revlon, New York, NY), except for a 2- $\times$ 4-mm window of exposed enamel. Each specimen was photographed to record the position of the enamel window for later use. MI Varnish (GC America Inc) was applied to the enamel windows of the experimental

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-05-16 via free access

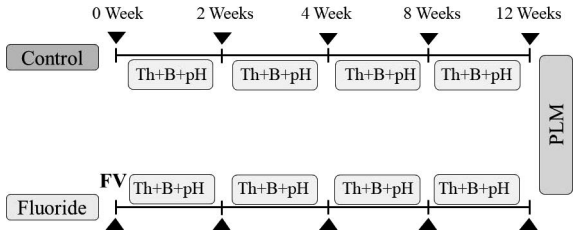

Figure 1. Illustration of the study design. ▲, FluoreCam imaging; FV, CPP-ACP fluoride varnish applied to the experimental group; Th+B+pH, thermocycling, brushing, and pH cycling; PLM, polarized light microscope.

group. It was scraped from the enamel windows on the following day because, clinically, varnish usually starts to abrade off of smooth enamel surfaces during the first 24 hours. The control group received no treatment. Both groups were kept in 10 mL of artificial saliva (1.5 mmol/L Ca, 0.9 mmol/L P, 150 mmol/L KCl, 0.05 μg F/mL in 0.1 mol/L Tris buffer [pH 7.0]) for 24 hours.

Thermocycling and Toothbrushing

To simulate the thermal conditions of the oral environment, both groups were thermocycled.[25] The specimens were immersed alternately into two baths of distilled water (5°C and 55°C) for 15 seconds (LAUDA-Brinkmann LP, Delran, NJ),[25] with a 5-second transfer time at 23°C. A total of 150, 300, 600, and 1200 cycles were performed, representing 2, 4, 8, and 12 weeks, respectively (10 cycles/d).[25] To simulate the mechanical effect of toothbrushing, the groups were placed in a toothbrushing simulator (Proto-Tech Oral Wear products, Portland, Ore).[26] The specimens were individually placed in customized rubber molds (Exaflex Putty, GC America, Inc) mounted in the brushing simulator. Medium-bristled toothbrushes (Deluxe Denta-Brite, Eagle, NY) were centered over the exposed enamel windows and oriented to brush in a mesiodistal direction.[26] To simulate a normal manual brushing force, a constant force of 280 g and 20 strokes/d was applied. A total of 300, 600, 1200, and 1800 strokes were applied for simulating 2, 4, 8, and 12 weeks, respectively.[26] A slurry of fluoridated toothpaste (Crest, Procter and Gamble, Cincinnati, Ohio), with a 1:3 paste to water ratio, was constantly circulated during the brushing procedure.[26] New brushes and fresh slurry solution were used for each group.

pH Cycling

The two groups were subjected to 9 days of pH cycling (8-day de/remineralization +1-day remineralization).[27] Falcon tubes (VWR, Radnor, Pa) were prepared filled with demineralizing solution (50 mL) and remineralizing solution (25 mL). The specimens were immersed alternately in demineralizing solution for 4 hours and remineralizing solution for 20 hours over an 8-day period.[27] The tubes were kept in an incubator at 37°C and under constant agitation (Excella E24 Incubator Shaker Series, New Brunswick Scientific Co, Inc, Enfield, Conn). The solutions were replaced every 4 days. On day 9, the specimens were kept in the remineralizing solution for 24 hours. The demineralizing solution consisted of 0.05 M acetate buffer containing 1.28 mmol/L Ca, 0.74 mmol/L P, and 0.03 μg F/mL (pH 5), prepared from Ca $(NO_3)_2.4\ H_2O$, KH_2PO_4, and NaF, respectively. The proportion of demineralizing solution per area of exposed enamel was 6.25 mL/mm^2. The remineralizing solution consisted of 1.5 mmol/L Ca, 0.9 mmol/L P, 150 mmol/L KCl, 0.05 μg F/mL in 0.1 mol/L Tris buffer (pH 7.0). The proportion of remineralizing solution per area of exposed enamel was 12 mL/mm^2. Following pH cycling, the nail polish was removed because it could affect the FluoreCam readings. The nail polish was reapplied, leaving the same enamel window exposed, and the subsequent thermocycling and toothbrushing were conducted.

PLM Sample Preparation

To ensure consistency of pattern, five specimens from each group were randomly selected and prepared for PLM assessments. The roots were removed, and the crowns were serially sectioned perpendicular to the 2- × 4-mm enamel windows. The slices were ground and polished to a thickness of approximately 100 μm and soaked in distilled water overnight. The demineralized enamel lesions were examined under the PLM attached to a camera (Olympus-BX51, Olympus Corp, Center Valley, Pa). The mean lesion depths of the demineralized enamel were measured in microns using CellSens standard software (Olympus Corp). Three lines were drawn, one at the center and one on each side of the lesion (ie, enamel window). Each line was perpendicular to the outer enamel surface and extended to the depth of the lesion.

Statistical Analysis

The data were normally distributed. Independent *t* tests were used to evaluate pretreatment differences. Repeated-measure analysis of variance, with Greenhouse-Geisser Correction, was used to determine the effects of time and varnish. The effects of time within each group were then tested using paired *t* tests with Bonferroni corrections. Between-group differences at 2, 4, 8, and 12 weeks were conducted using independent *t* tests with Bonferroni corrections.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-05-16 via free access

Table 1. Mean Changes in Area, Intensity, and Impact of Enamel Demineralization Over Time for the Control and Varnish Group, Along With Probabilities From Paired *t* Test

		0–2		2–4		4–8		8–12	
Output	Group	Mean	*P* Value	Mean	*P* Value	Mean	*P* Value	Mean	*P* Value
Area	Control	2.22	<.001	1.46	<.001	1.08	<.001	0.68	<.001
	Fluoride	−0.70	<.001	0.72	<.001	1.17	<.001	0.92	<.001
Intensity	Control	−1.63	<.001	−1.45	<.001	−1.87	<.001	−1.73	<.001
	Fluoride	0.05	.72	−0.13	.20	−1.03	<.001	−1.20	<.001
Impact	Control	−28.60	<.001	−26.37	<.001	−30.54	<.001	−27.63	<.001
	Group	5.22	<.005	−4.82	.003	−15.09	<.001	−16.52	<.001

RESULTS

FluoreCam

At pretreatment, there were no statistically significant between-group differences for area, intensity, or impact. Posttreatment, there were statistically significant ($P < .001$) time and varnish effects for area, intensity, and impact. In the control group, area, intensity, and impact of demineralization showed statistically significant increases at every time point (Table 1; Figures 2–4).

In the experimental group, the area of demineralized enamel decreased significantly after 2 weeks. After 4 weeks, there was no significant difference in area compared with pretreatment. There was an increase in area ($P = 0.001$) after 8 weeks, which continued until 12 weeks ($P < .001$; Figure 2).

The intensity of demineralization in the experimental group showed no statistically significant changes after 2 weeks or 4 weeks. There was a statistically significant increase in the intensity of demineralization after 8 and 12 weeks (Figure 3).

The impact of demineralization in the experimental group followed the same pattern as the area of demineralization. The impact decreased significantly after 2 weeks. After 4 weeks, there was no significant difference compared with pretreatment, followed by significant increases at 8 and 12 weeks (Figure 4).

There were significant between-group differences in area, intensity, and impact at each of the time periods (Figures 2, 3, and 4).

Polarized Light Microscopy

The PLM images revealed typical WSLs in the untreated control representative samples. The mean lesion depth was 190 ± 34 μm. The experimental group showed more limited areas of enamel demineralization, with a mean lesion depth of 37 ± 9 μm.

DISCUSSION

In the present study, untreated control enamel exposed to pH cycling demineralized over time, but the rate of demineralization decelerated. Demineralization of the control group increased 127% during the first 4 weeks, 71% during the next 4 weeks, and 62% during the last 4 weeks, confirming that demineralized enamel is less likely to demineralize than sound enamel.[23] This explains why WSL development is greatest during the first 6 months of orthodontic treatment.[28] Deceleration of demineralization might be attributed to the formation of fluoridated hydroxyapatite (HA) crystals that are less soluble and more resistant to further acid attacks than the original HA crystals.[29] At a low pH, acidity removes surface impurities that increase surface area and expose more reactive HA crystals.[29] When the pH neutralizes, the partially

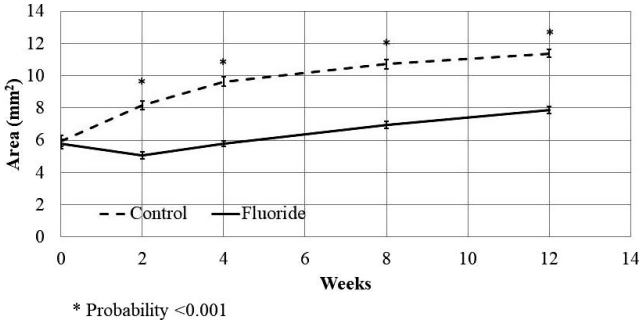

Figure 2. Changes in area of enamel demineralization over time in the control and varnish groups, along with probabilities from independent *t* test.

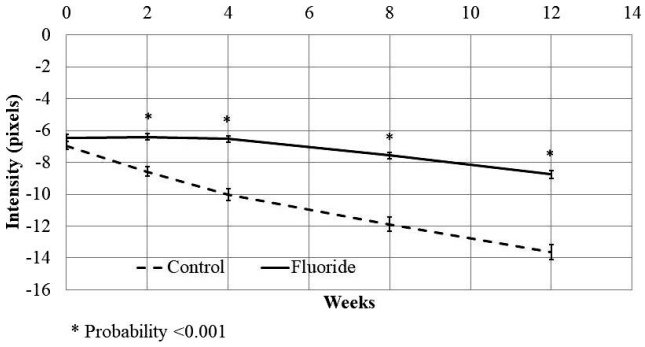

Figure 3. Changes in the intensity of enamel demineralization over time in the control and varnish groups, along with probabilities from independent *t* test.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-05-16 via free access

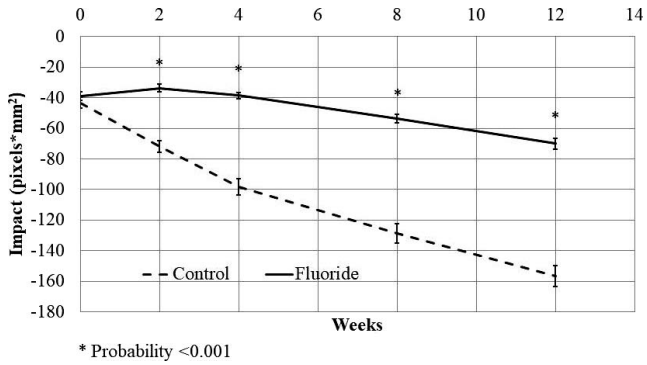

Figure 4. Changes in the impact of enamel demineralization over time in the control and varnish groups, along with probabilities from independent *t* test.

demineralized HA crystals uptake calcium, phosphate, and fluoride ions to form fluoridated HA crystals, which precipitate on enamel.

CPP-ACP fluoride varnish initially remineralizes enamel. In the present study, FluoreCam baseline readings and images showed minor demineralization areas. The enamel remineralized during the first 2 weeks after fluoride varnish application despite thermal (thermocycling), chemical (pH cycling), and mechanical (brushing) challenges. This led to a 12% decrease in the area, a 1.5% decrease in intensity, and a 14% decrease in the impact of demineralization. Knosel et al.,[30] who compared the effect of different varnishes using quantitative light-induced fluorescence, showed no enamel demineralization or remineralization during the first 2 weeks.[30] However, the varnish they tested did not contain CPP-ACP. CPP-ACP buffers free calcium and phosphate ions, maintaining a state of supersaturation with respect to enamel HA, which facilitates remineralization and prevents demineralization.[9,18]

Enamel demineralization begins approximately 2 weeks after CPP-ACP fluoride varnish application. After 4 weeks, the enamel mineralization returned to baseline values, indicating a 100% varnish preventive effect. Knosel and colleagues[30] also showed a 100% preventive effect 2 and 4 weeks after application. However, the enamel in their study was not subjected to thermal cycling and mechanical forces, and their enamel specimens were exposed to shorter cycles of demineralization (30 min/d) than the present study (4 h/d). These factors possibly increased the loss of the loosely bonded fluoride (CaF_2) but not the fluoroapatite and thus provided 100% enamel protection.

After 8 and 12 weeks, the preventive effect of CPP-ACP fluoride diminished to 75% and 50%, respectively. Therefore, demineralization must have begun sometime between 4 and 8 weeks. A previous clinical trial that applied a sodium fluoride varnish every 6 weeks reported only a 30% decrease in WSLs compared with a placebo application.[11] The effect of the fluoride varnish probably starts to diminish after 6 weeks because most of the fluoride bonded to the enamel surface is loosely bonded (CaF_2). It gradually debonds over time under normal oral conditions.[8] CPP-ACP fluoride varnish may be more effective over a slightly longer period of time than other varnishes.

Importantly, demineralization 12 weeks after fluoride varnish application was comparable to the demineralization after 2 weeks in the control group. The fluoride varnish used in the present study might be more effective than other varnishes because it contains CPP-ACP.[17] MI varnish has the highest cumulative fluoride release (303 μg/mL)[31] and the greatest cumulative calcium and phosphate release.[16]

CONCLUSIONS

Within the limitations of this in vitro study, it can be concluded that CPP-ACP fluoride varnish should be considered for routine clinical use to prevent WSLs in orthodontics because:

- CPP-ACP fluoride varnish provides a net remineralization for up to 2 weeks after application.
- The preventive effect of the fluoride varnish is 100% for at least 4 weeks; therefore, clinical application should be administered within this time interval.
- The overall demineralization that occurs 12 weeks after varnish application is equivalent to 2-week demineralization if the varnish was not applied.

REFERENCES

1. Sudjalim TR, Woods MG, Manton DJ. Prevention of white spot lesions in orthodontic practice: a contemporary review. *Aust Dent J*. 2006;51:284–289.
2. Chapman JA, Roberts WE, Eckert GJ, Kula KS, Gonzalez-Cabezas C. Risk factors for incidence and severity of white spot lesions during treatment with fixed orthodontic appliances. *Am J Orthod Dentofacial Orthop*. 2010;138:188–194.
3. Julien KC, Buschang PH, Campbell PM. Prevalence of white spot lesion formation during orthodontic treatment. *Angle Orthod*. 2013;83:641–647.
4. Brown MD, Campbell PM, Schneiderman ED, Buschang PH. A practice-based evaluation of the prevalence and predisposing etiology of white spot lesions. *Angle Orthod*. 2016;86:181–186.
5. Heymann GC, Grauer D. A contemporary review of white spot lesions in orthodontics. *J Esthet Restor Dent*. 2013;25:85–95.
6. Benson PE, Parkin N, Dyer F, et al. Fluorides for the prevention of early tooth decay (demineralised white lesions) during fixed brace treatment. *Cochrane Database Syst Rev*. 2013;12:CD003809.
7. ten Cate JM, Featherstone JD. Mechanistic aspects of the interactions between fluoride and dental enamel. *Crit Rev Oral Biol Med*. 1991;2:283–296.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-05-16 via free access

8. Retief DH, Sorvas PG, Bradley EL, Taylor RE, Walker AR. In vitro fluoride uptake, distribution and retention by human enamel after 1- and 24-hour application of various topical fluoride agents. *J Dent Res*. 1980;59:573–582.
9. Weyant RJ, Tracy SL, Anselmo TT, et al. Topical fluoride for caries prevention. *J Am Dent Assoc*. 2013;144:1279–1291.
10. Farhadian N, Miresmaeili A, Eslami B, Mehrabi S. Effect of fluoride varnish on enamel demineralization around brackets: an in-vivo study. *Am J Orthod Dentofacial Orthop*. 2008; 133(4 suppl):S95–S98.
11. Stecksen-Blicks C, Renfors G, Oscarson ND, Bergstrand F, Twetman S. Caries-preventive effectiveness of a fluoride varnish: a randomized controlled trial in adolescents with fixed orthodontic appliances. *Caries Res*. 2007;41:455–459.
12. Gomez J, Tellez M, Pretty IA, Ellwood RP, Ismail AI. Non-cavitated carious lesions detection methods: a systematic review. *Community Dent Oral Epidemiol*. 2013;41:54–66.
13. Bilgin G, Yanikoglu F, Tagtekin D, Stookey GK, BRS. Remineralization potential of different agents and assessment by a new caries detection device. *Gavin J Dent Sci*. 2016;2016(1):5.
14. Zero DT. Dental caries process. *Dent Clin North Am*. 1999; 43:635–664.
15. Reynolds E. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. *J Dent Res*. 1997;76:1587–1595.
16. Cochrane NJ, Shen P, Yuan Y, Reynolds EC. Ion release from calcium and fluoride containing dental varnishes. *Aust Dent J*. 2014;59:100–105.
17. Tuloglu N, Bayrak S, Tunc ES, Ozer F. Effect of fluoride varnish with added casein phosphopeptide-amorphous calcium phosphate on the acid resistance of the primary enamel. *BMC Oral Health*. 2016;16:103.
18. Reynolds EC, Cain CJ, Webber FL, et al. Anticariogenicity of calcium phosphate complexes of tryptic casein phosphopeptides in the rat. *J Dent Res*. 1995;74:1272–1279.
19. Schupbach P, Neeser JR, Golliard M, Rouvet M, Guggenheim B. Incorporation of caseinoglycomacropeptide and caseinophosphopeptide into the salivary pellicle inhibits adherence of mutans streptococci. *J Dent Res*. 1996;75: 1779–1788.
20. Reynolds EC, Cai F, Shen P, Walker GD. Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum. *J Dent Res*. 2003;82:206–211.
21. Poggio C, Lombardini M, Dagna A, Chiesa M, Bianchi S. Protective effect on enamel demineralization of a CPP-ACP paste: an AFM in vitro study. *J Dent*. 2009;37:949–954.
22. Reynolds EC, Cai F, Cochrane NJ, et al. Fluoride and casein phosphopeptide-amorphous calcium phosphate. *J Dent Res*. 2008;87:344–348.
23. Abufarwa M, Voorhees RD, Varanasi VG, Campbell PM, Buschang PH. White spot lesions: does etching really matter? *J Investig Clin Dent*. 2018;9(1).
24. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 2007;39:175–191.
25. Brown W, Jacobs H, Thompson R. Thermal fatigue in teeth. *J Dent Res*. 1972;51:461–467.
26. Buren JL, Staley RN, Wefel J, Qian F. Inhibition of enamel demineralization by an enamel sealant, Pro Seal: an in-vitro study. *Am J Orthod Dentofacial Orthop*. 2008;133(4 suppl): S88–S94.
27. Queiroz CS, Hara AT, Paes Leme AF, Cury JA. pH-cycling models to evaluate the effect of low fluoride dentifrice on enamel de- and remineralization. *Braz Dent J*. 2008;19(1): 21–27.
28. Tufekci E, Dixon JS, Gunsolley JC, Lindauer SJ. Prevalence of white spot lesions during orthodontic treatment with fixed appliances. *Angle Orthod*. 2011;81:206–210.
29. Al-Khateeb S, Exterkate R, Angmar-Mansson B, ten Cate JM. Effect of acid-etching on remineralization of enamel white spot lesions. *Acta Odontol Scand*. 2000;58:31–36.
30. Knosel M, Forslund L, Jung K, Ziebolz D. Efficacy of different strategies in protecting enamel against demineralization during fixed orthodontic treatment. *J Orofac Orthop*. 2012; 73:194–203.
31. Al Dehailan L, Martinez-Mier EA, Lippert F. The effect of fluoride varnishes on caries lesions: an in vitro investigation. *Clin Oral Investig*. 2016;20:1655–1662.

Downloaded from https://prime-pdf-watermark.prime-prod.pubfactory.com/ at 2025-05-16 via free access