INTRODUCTION

Dental caries is a widespread, chronic, and infectious disease affecting the hard tissues of the teeth. *Streptococcus mutans* is an important bacterium responsible for the development of dental caries.[1] The application of topical fluoride plays a significant role in the control of caries lesions. Accordingly, the utilization of varnishes has been demonstrated to be beneficial in the preschool children.[2]

Varnishes are easy to apply, safe, and well-accepted by patients also not sensitive to moisture and can harden even under saliva.[3] The long-term prophylactic effect of varnishes depends on the sustained release of fluoride.[4,5]

Another remineralization effect of fluoride is its ability to reduce acid formation by *S. mutans* in dental plaques or reduction in *S. mutans* count in saliva.[6,7] Since the sole use of fluoride varnishes had little effect on the total salivary levels of *S. mutans*, they were suggested to be used in combination with other antimicrobial agents.[8] Xylitol is a sugar alcohol derived primarily from the forest and agricultural materials.[9] According to several studies, xylitol intake leads to positive outcomes such as reduced incidence of caries and *S. mutans* levels in oral flora due to the inability of five-carbon sugar xylitol to be fermented by *S. mutans*.[9,10] and xylitol varnishes can be promising alternatives to increase enamel remineralization.[11]

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is a novel agent for the prevention of dental caries and xylitol varnish is promising alternatives to increase enamel remineralization.

Objective: Different fluoride varnishes are used for the prevention of dental caries. The aim of this study was to compare the antimicrobial effect of different fluoride varnishes, containing xylitol and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), on *Streptococcus mutans*.

Materials and Methods: In this in vitro study, the antibacterial effects of four varnishes, namely Polimo and V‑varnish (containing xylitol), MI varnish (containing CPP‑ACP), and Preventa, were evaluated against *S. mutans*. The disc diffusion method was used for testing the bacterial sensitivity. The data were analyzed using the Kruskal–Wallis and Mann–Whitney U‑tests.

Results: According to the results, Polimo showed the highest antibacterial effects, compared to the other three varnishes (*P* ≤ 0.05). Growth inhibition zones were not observed in V‑varnish and Preventa. The mean diameter of inhibition zone around the MI varnish was significantly higher, compared to those of the V varnish and Preventa (*P* ≤ 0.05).

Conclusion: As the findings indicated, the fluoride varnish containing xylitol and CPP-ACP could be more effective in the prevention of dental caries.

Keywords: Casein phosphopeptide-amorphous calcium phosphate, fluoride varnish, *Streptococcus mutans*, xylitol

Access this article online

Quick Response Code:

Website: www.jispcd.org

DOI: 10.4103/jispcd.JISPCD_67_18

Address for correspondence: Dr. Somayeh Hekmatfar, Dental Faculty, Ardabil University of Medical Sciences, Ardabil, Iran.

E-mail: hekmatfar24@gmail.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Jafari K, Hekmatfar S, Fereydunzadeh M. *In vitro* comparison of antimicrobial activity of conventional fluoride varnishes containing xylitol and casein phosphopeptide-amorphous calcium phosphate. J Int Soc Prevent Commun Dent 2018;8:309-13.
caries. This milk product facilitates remineralization, inhibits demineralization, and prevents dental caries by forming a calcium phosphate reservoir. Furthermore, CPP incorporates into the salivary pellicle and thereby substantially reduces the adhesion of S. mutans.\textsuperscript{[12,13]} Recently, several types of these agents have been introduced such as fluoride varnishes, pastes, and dentifrices. Applications of CPP-ACP paste as an adjunct to standard oral hygiene reduced the size and degree of demineralization of white spot lesions in clinical study.\textsuperscript{[14,15]}

A potential synergistic effect of CPP-ACP and fluoride therapy is also disputed. Previous study has reported that the addition of CPP-ACP to fluoride varnish increased the acid resistance of primary enamel than other fluoride varnishes.\textsuperscript{[16]} Mohd Said compared remineralization potential of topical fluoride varnishes with added calcium phosphate-based delivery systems on artificial enamel caries and observed the effect of topical fluoride varnishes containing 5% sodium fluoride and CPP-ACP, xylitol-coated calcium and phosphate, and ACP do not achieve better remineralization of artificial enamel carious lesions when compared with 5% sodium fluoride alone.\textsuperscript{[17]}

With this background in mind, the present \textit{in vitro} study was conducted to investigate the antibacterial effectiveness of four fluoride varnishes with different compositions on \textit{S. mutans}. Knowledge on the antimicrobial effect of different fluoride varnishes can help in choosing most effective varnishes for clinical applications.

**Materials and Methods**

In this \textit{in vitro} study, the antibacterial effects of four fluoride varnishes, namely Polimo, V-varnish (fluoride varnish containing xylitol), MI varnish (fluoride varnish containing CPP-ACP), and Preventa, were evaluated against \textit{S. mutans} (ATCC 35668) [Table 1]. The bacteria were provided by the Iranian Research Organization for Science and Technology, Tehran, Iran, and prepared in lyophilized form. To confirm the strain identity, the laboratory tests of microbiology were conducted in the Microbiology Laboratory of Ardabil University of Medical Sciences, Ardabil, Iran.

The bacterial sensitivity was tested using the disc diffusion method. In this technique, when the medicinal compound penetrates in agar from the disc borders, its concentration is progressively reduced to the extent that it has no more power to prevent bacterial growth. Therefore, bacterial growth stops in the penetrating zone of the drug compounds. Accordingly, the diameter of the inhibition zone specifies the relative susceptibility of bacteria to specific material compound.

To implement this method, the fresh culture of \textit{S. mutans} at the turbidity of 0.5 McFarland standards was spread on Hinton agar culture medium. The sterile paper discs (Blank disc, PadTanTeb, Iran) were coated with the fluoride varnishes (20 µl) and transferred in the plates containing the media. In the next step, the culture media in an anaerobic jar containing gasp (A) were used to drain the culture medium for \textit{S. mutans}. Finally, the anaerobic jar containing the culture media was incubated at 37°C for 24 h. Subsequently, the sensitivity of \textit{S. mutans} strain to varnish fluoride was evaluated by measuring the diameter of the inhibition zone.

Blank disc that was empty and penicillin disc (antibacterial medicine, Iran) was placed on the plate surfaces as negative and positive controls, respectively. Each experiment was repeated 10 times, and the mean and standard deviation were calculated. The data were analyzed using the Kruskal–Wallis and Mann–Whitney U-tests.

**Results**

In the present study, out of the four investigated varnishes, Polimo and MI fluoride varnishes showed antibacterial effects [Table 2]. Polimo had the maximum area of no growth around the discs and highest diameter of the inhibition zones. This study revealed a significant difference between Polimo and other varnishes in

**Table 1: Fluoride varnishes selected for this study**

| Product       | Manufacturer           | Composition                                                                 |
|---------------|------------------------|-----------------------------------------------------------------------------|
| MI varnish    | GC, Tokyo, Japan       | 30%-50% polyvinyl acetate, 10%-30% hydrogenated rosin, 20%-30% ethanol, 1%-8% sodium fluoride, 1%-5% CPP-ACP, 1%-5% silicon dioxide |
| Preventa      | Asia chemi teb, Iran   | 5% sodium fluoride, resin, alcohol                                          |
| V-varnish     | Vericon, Korea         | Sodium fluoride, rosin, 20% ethanol, 17% xylitol, tricalcium phosphate      |
| Polimo fluoride varnish | Imicryl, Turkey | 5% sodium fluoride, xylitol, flavor                                        |

**Table 2: Mean diameter of inhibition zone around the discs coated with fluoride varnishes (mm)**

| Product       | Mean  | SD   | P    |
|---------------|-------|------|------|
| V-varnish     | 0.00  | 0.00 | <0.05|
| Preventa      | 0.00  | 0.00 |      |
| Polimo        | 10.10 | 0.56 |      |
| MI varnish    | 1.95  | 1.61 |      |

SD=Standard deviation
terms of the antibacterial effect \( (P \leq 0.05) \). The mean diameter of inhibition zone around the MI varnish was significantly higher than those of the Preventa and V-varnish \( (P \leq 0.05) \).

**Discussion**

According to several studies, the use of fluoride varnishes is effective in caries prevention among children.\(^2\) Topical fluoride interacts with saliva and forms calcium fluoride (CaF\(_2\)) compounds on enamel. CaF\(_2\) is stabilized by pellicle proteins and secondary phosphate at neutral pH. When the pH of plaque drops, CaF\(_2\) begins to dissolve and release fluoride ions; therefore, it acts as a prolonged source of fluoride after application.\(^{18-20}\)

The most important anticaries effects of fluoride are the inhibition of demineralization and enhancement of remineralization of early caries lesions.\(^2\) In addition, fluoride can inhibit acid production by bacteria and may reduce the number of \textit{S. mutans}.\(^{6,21}\) Recently, a range of fluoride varnishes with different compositions have been developed to enhance the caries prevention.

It is well established that xylitol has antimicrobial activity against \textit{S. mutans} in the oral cavity and CPP-ACP increases the retention of fluoride and calcium ions in the oral environment and improves the remineralization of early lesions.\(^{22,23}\)

In our study, we investigated and compared the antimicrobial effect of four dental varnishes. Different methods have been used in the literatures to assess the antibacterial activity of restorative materials. The main and very common method is disc diffusion test.\(^{24}\) In our study, we also applied this method to testing antibacterial activity of fluoride varnishes. Polimo showed a significantly higher antimicrobial activity against \textit{S. mutans} as compared to the other fluoride varnishes. This difference may be explained by the composition of the varnishes and their mechanisms of action.

The usefulness of xylitol in the prevention of dental caries, especially primary caries, has been documented.\(^{25,26}\) Vongsavan demonstrated that the combination of fluoride and xylitol varnishes was beneficial for the prevention of enamel demineralization; however, the addition of xylitol to fluoride varnish resulted in no significant better than fluoride varnish alone in vitro.\(^{27}\)

Emamieh studied the clinical antibacterial effects of CPP-ACP xylitol chewing gum. They observed that \textit{S. mutans} in saliva significantly reduced in both groups after the consumption of the chewing gums. Nevertheless, in the mentioned study, the rate of \textit{S. mutans} in saliva was significantly higher in the xylitol group, compared to that in the CPP-ACP group.\(^{28}\)

In the current study, we used two fluoride varnishes containing xylitol (i.e., V-varnish and Polimo). Polimo demonstrated higher antimicrobial effects; however, the inhibition zone was not observed in V-varnish. V-varnish has a higher viscosity than the other test materials, which may have resulted in the diffusion on the plate surfaces. Regarding this, it is suggested to investigate the composition of V-varnish.

MI varnish is consistent with the bioavailable nature of CPP-ACP contained within the varnish. The CPP-ACP derived from milk protein casein has been reported to reduce the demineralization of the tooth structure and enhance remineralization.\(^{29,30}\) The synergistic effect of CPP-ACP with the fluoride present in MI varnish showed lower demineralization and higher remineralization potentials.\(^{31}\) Our results were in accordance with the clinical study done by Patel that stated the varnish containing fluoride with CPP-ACP to be effective in reduction of salivary \textit{S. mutans} count in comparison to fluoride or chlorhexidine varnish. This result could be due to the additive anticariogenic effect of CPP-ACP and fluoride attributable to localization of ACPF at the tooth surface by the CPP.\(^{19}\)

Erdem reported a reduction in the bacterial viability of \textit{S. mutans} in biofilm after the application of CPP-ACP; nonetheless, this reduction was not statistically significant.\(^3\) Our findings are consistent with the results of a recent \textit{in situ} study that demonstrated a delay in plaque (biofilm) formation on the germanium surfaces treated with CPP-ACP, compared to the untreated ones.\(^{32}\) CPP-ACP has been shown to bind to \textit{S. mutans}. This binding is likely to be mediated by calcium cross-linking of cell surface phosphate moieties, as well as by hydrophobic and hydrogen bond-mediated interactions.\(^{14,33}\)

In a study investigating the relationship between fluoride concentrations and antibacterial effects, it was revealed that the low fluoride concentration was associated with increased viable bacterial counts.\(^{31}\) In our study, the fluoride concentrations in all group were similar. The difference in their composition may account for their varied antimicrobial effects.

Growth inhibition zones were not observed in V-varnish and Preventa. This finding requires further discussion over the antibacterial effects of fluoride varnishes. However, fluoride may interfere with bacterial metabolism and inhibit bacterial growth.\(^{5,34}\) The results of this study showed the limited antibacterial effect of fluoride. Further investigations are needed to confirm these results and develop strategies for using such products to prevent dental caries.
One limitation of this study may include using of only S. mutans while the other pathogenic bacteria are associated with the dental biofilms in oral environment. Within the limitations of the in vitro condition, study under clinical situations in children is required to confirm the data obtained from this study.

**CONCLUSION**

As the findings indicated, all fluoride varnishes, except for V-varnish and Preventa, had antimicrobial effect. Among the four evaluated fluoride varnishes, Polimo then MI varnish showed the highest antibacterial effect against S. mutans. Consequently, the application of Polimo and MI varnish not only inhibited demineralization and enhanced remineralization but also reduced the level of S. mutans in the oral cavity.

**ACKNOWLEDGMENT**

This study was part of a doctoral thesis (62#). The authors would like to express their gratitude to the Research Deputy of Ardabil University of Medical Sciences, Ardabil, Iran, for financially supporting this study.

**FINANCIAL SUPPORT AND SPONSORSHIP**

Nil.

**CONFLICTS OF INTEREST**

There are no conflicts of interest.

**REFERENCES**

1. Fejerskov O, Kidd EA, editors. Clinical cariology and operative dentistry in the twenty-first century. In: Dental Caries: The Disease and its Clinical Management. Denmark, Copenhagen: Blackwell Publishing Ltd.; 2003. p. 179-88.
2. Marinho VC, Worthington HV, Walsh T, Clarkson JE. Fluoride varnishes for preventing dental caries in children and adolescents. Cochrane Database Syst Rev 2013;CD002279. doi: 10.1002/14651858.
3. Pinar Erdem A, Sepet E, Kulekci G, Trosola SC, Guven Y. Effects of two fluoride varnishes and one fluoride/chlorhexidine varnish on Streptococcus mutans and Streptococcus sobrinus biofilm formation in vitro. Int J Med Sci 2012;9:129-36.
4. Scheie AA. The role of antimicrobials. In: Fejerskov O, Kidd E, editors. Dental Caries: The Disease and its Clinical Management. Denmark, Copenhagen: Blackwell Publishing Ltd.; 2003. p. 179-88.
5. Horowitz HS, Ismail AI. Topical fluorides in caries prevention. In: Fejerskov O, Ekstrand J, Burt BA, editors. Fluoride in Dentistry, 2nd ed. Copenhagen: Munksgaard; 1996. p. 311-27.
6. Badatia S, Badatia RG, Thanveer K, Krishnan AC. Effects of fluoride varnish on Streptococcus mutans count in saliva. Int J Clin Pediatr Dent 2017;10:62-6.
7. Chau NP, Pandit S, Jung JE, Jeon JG. Evaluation of Streptococcus mutans adhesion to fluoride varnishes and subsequent change in biofilm accumulation and acidogenicity. J Dent 2014;42:726-34.
8. Söderling E, Isokangas P, Pienilähtinen K, Tenouvo J, Alanen P. Influence of maternal xylitol consumption on mother-child transmission of mutans streptococci: 6-year follow-up. Caries Res 2001;35:173-7.
9. Milgrom P, Ly KA, Roberts MC, Rothen M, Mueller G, Yamaguchi DK, et al. Mutans streptococci dose response to xylitol chewing gum. J Dent Res 2006;85:177-81.
10. Jannesson L, Rentver S, Kjellslodtter P, Gaffar A, Nabi N, Birkhed D, et al. Effect of a triclosan-containing toothpaste supplemented with 10% xylitol on mutants streptococci in saliva and dental plaque. A 6-month clinical study. Caries Res 2002;36:36-9.
11. Cardoso CA, Cassiano LP, Costa EN, Souza-E-Silva CM, Magalhães AC, Grizzo LT, et al. Effect of xylitol varnishes on remineralization of artificial enamel caries lesions in situ. J Dent 2016;50:74-8.
12. Reynolds EC. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. J Dent Res 1997;76:1587-95.
13. 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;82:206-11.
14. Memarpour M, Fakhraei E, Dadaein S, Vosoughi M. Efficacy of fluoride varnish and casein phosphopeptide-amorphous calcium phosphate for remineralization of primary teeth: A randomized clinical trial. Med Princ Pract 2015;24:231-7.
15. Güçlü ZA, Alaçam A, Coleman NJ. A 12-week assessment of the treatment of white spot lesions with CPP-ACP paste and/or fluoride varnish. Biomed Res Int 2016;2016:8357621.
16. Tüloğlu N, Bayrak S, Tunc ES, Özer 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.
17. Mohd Said SN, Ekmabaram M, Yiu CK. Effect of different fluoride varnishes on remineralization of artificial enamel carious lesions. Int J Paediatr Dent 2017;27:163-73.
18. Beerens MW, van der Veen MH, van Beek H, ten Cate JM. Effects of casein phosphopeptide amorphous calcium fluoride phosphate paste on white spot lesions and dental plaque after orthodontic treatment: A 3-month follow-up. Eur J Oral Sci 2010;118:610-7.
19. Patel PM, Hugar SM, Halikerimuth S, Badakar CM, Gokhale NS, Thakkar PJ, et al. Comparison of the effect of fluoride varnish, chlorhexidine varnish and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) varnish on salivary Streptococcus mutans level: A six month clinical study. J Clin Diagn Res 2017;11:ZC53-9.
20. Duangthip D, Chu CH, Lo EC. A randomized clinical trial on arresting dentine caries in preschool children by topical fluorides–18 month results. J Dent 2016;44:57-63.
21. Lynch RJ, Navada R, Walia R. Low-levels of fluoride in plaque and saliva and their effects on the demineralisation and remineralisation of enamel; role of fluoride toothpastes. Int Dent J 2004;54:304-9.
22. Cochrane NJ, Cai F, Huq NL, Burrow MF, Reynolds EC. New approaches to enhanced remineralization of tooth enamel. J Dent Res 2010;89:1187-97.
23. Vogel GL. Oral fluoride reservoirs and the prevention of dental caries. Monogr Oral Sci 2011;22:146-57.
24. Farrugia C, Camilleri J. Antimicrobial properties of conventional restorative filling materials and advances in antimicrobial properties of composite resins and glass ionomer cements – A literature review. Dent Mater 2015;31:e89-99.
25. Söderling E, Alaräisänen L, Scheinin A, Mäkinen KK. Effect of xylitol and sorbitol on polysaccharide production by and adhesive properties of Streptococcus mutans. Caries Res 1987;21:109-16.
26. Suda R, Suzuki T, Takiguchi R, Egawa K, Sano T, Hasegawa K, et al. The effect of adding calcium lactate to xylitol chewing gum on remineralization of enamel lesions. Caries Res 2006;40:43-6.
27. Vongsavan K, Surarit R, Rirattanapong P. The combined effect of xylitol and fluoride in varnish on bovine teeth surface microhardness. Southeast Asian J Trop Med Public Health 2014;45:505-10.
28. Emamieh S, Khaterizadeh Y, Goudarzi H, Ghasemi A, Baghban AA, Torabzadeh H, et al. The effect of two types chewing gum containing casein phosphopeptide-amorphous calcium phosphate and xylitol on
salivary *Streptococcus mutans.* J Conserv Dent 2015;18:192-5.

29. Cai F, Shen P, Morgan MV, Reynolds EC. Remineralization of enamel subsurface lesions *in situ* by sugar-free lozenges containing casein phosphopeptide-amorphous calcium phosphate. Aust Dent J 2003;48:240-3.

30. Iijima Y, Cai F, Shen P, Walker G, Reynolds C, Reynolds EC, *et al.* Acid resistance of enamel subsurface lesions remineralized by a sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. Caries Res 2004;38:551-6.

31. Duraisamy V, Xavier A, Nayak UA, Reddy V, Rao AP. *An in vitro* evaluation of the demineralization inhibitory effect of F(⁻) varnish and casein phosphopeptide-amorphous calcium phosphate on enamel in young permanent teeth. J Pharm Bioallied Sci 2015;7:S513-7.

32. Rahiotis C, Vougiouklakis G, Eliades G. Characterization of oral films formed in the presence of a CPP-ACP agent: *An in situ* study. J Dent 2008;36:272-80.

33. Allaker RP. The use of nanoparticles to control oral biofilm formation. J Dent Res 2010;89:1175-86.

34. Bradshaw DJ, Marsh PD, Hodgson RJ, Visser JM. Effects of glucose and fluoride on competition and metabolism within *in vitro* dental bacterial communities and biofilms. Caries Res 2002;36:81-6.