

## *In vitro* Comparison of Antimicrobial Activity of Conventional Fluoride Varnishes Containing Xylitol and Casein Phosphopeptide-Amorphous Calcium Phosphate

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Received : 09-02-18.

Accepted : 23-05-18.

Published : 18-07-18.

### 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.<sup>[1]</sup> 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.<sup>[2]</sup>

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

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.<sup>[6,7]</sup> Since the sole use of fluoride varnishes had little effect on the

### ABSTRACT

**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 ( $P \leq 0.05$ ). 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 ( $P \leq 0.05$ ).

**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

total salivary levels of *S. mutans*, they were suggested to be used in combination with other antimicrobial agents.<sup>[8]</sup> Xylitol is a sugar alcohol derived primarily from the forest and agricultural materials.<sup>[9]</sup> 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*,<sup>[9,10]</sup> and xylitol varnishes can be promising alternatives to increase enamel remineralization.<sup>[11]</sup>

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is a novel agent for the prevention of dental

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**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 Communit Dent 2018;8:309-13.

Access this article online	
<b>Quick Response Code:</b> 	<b>Website:</b> www.jispcd.org
	<b>DOI:</b> 10.4103/jispcd.JISPCD_67_18

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*.<sup>[12,13]</sup> 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.<sup>[14,15]</sup>

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.<sup>[16]</sup> 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.<sup>[17]</sup>

With this background in mind, the present *in vitro* study was conducted to investigate the antibacterial effectiveness of four fluoride varnishes with different compositions on *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 *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 *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

**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 chemiteb, 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
CPP-ACP=Casein phosphopeptide-amorphous calcium phosphate		

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

	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

specifies the relative susceptibility of bacteria to specific material compound.

To implement this method, the fresh culture of *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 *S. mutans*. Finally, the anaerobic jar containing the culture media was incubated at 37°C for 24 h. Subsequently, the sensitivity of *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

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.<sup>[2]</sup> Topical fluoride interacts with saliva and forms calcium fluoride ( $\text{CaF}_2$ ) compounds on enamel.  $\text{CaF}_2$  is stabilized by pellicle proteins and secondary phosphate at neutral pH. When the pH of plaque drops,  $\text{CaF}_2$  begins to dissolve and release fluoride ions; therefore, it acts as a prolonged source of fluoride after application.<sup>[18-20]</sup>

The most important anticaries effects of fluoride are the inhibition of demineralization and enhancement of remineralization of early caries lesions.<sup>[2]</sup> In addition, fluoride can inhibit acid production by bacteria and may reduce the number of *S. mutans*.<sup>[6,21]</sup> 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 *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.<sup>[22,23]</sup>

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.<sup>[24]</sup> In our study, we also applied this method to testing antibacterial activity of fluoride varnishes. Polimo showed a significantly higher antimicrobial activity against *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.<sup>[25,26]</sup> 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*.<sup>[27]</sup>

Emamieh studied the clinical antibacterial effects of CPP-ACP xylitol chewing gum. They observed that *S. mutans* in saliva significantly reduced in both groups after the consumption of the chewing gums. Nevertheless, in the mentioned study, the rate of *S. mutans* in saliva was significantly higher in the xylitol group, compared to that in the CPP-ACP group.<sup>[28]</sup>

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.<sup>[29,30]</sup> The synergistic effect of CPP-ACP with the fluoride present in MI varnish showed lower demineralization and higher remineralization potentials.<sup>[31]</sup> 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 *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.<sup>[19]</sup>

Erdem reported a reduction in the bacterial viability of *S. mutans* in biofilm after the application of CPP-ACP; nonetheless, this reduction was not statistically significant.<sup>[3]</sup> Our findings are consistent with the results of a recent *in situ* study that demonstrated a delay in plaque (biofilm) formation on the germanium surfaces treated with CPP-ACP, compared to the untreated ones.<sup>[32]</sup> CPP-ACP has been shown to bind to *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.<sup>[14,33]</sup>

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.<sup>[31]</sup> 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.<sup>[5,34]</sup> 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.

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