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## **Antibacterial effect of amorphous calcium phosphate-casein phosphopeptide varnish against Streptococcus mutans: An exploratory *in vivo* randomized controlled trial**

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### **Abstract**

**Background:** Nowadays, amorphous calcium phosphate-casein phosphopeptide and tricalcium phosphate are being incorporated into fluoride varnishes to enhance remineralization potential which might interfere with antibacterial effect of fluoride in varnishes. Aim of study was to evaluate and compare the antimicrobial property of sodium fluoride varnish reinforced with amorphous calcium phosphate-casein phosphopeptide and tricalcium phosphate against streptococcus mutans.

**Methods:** In the clinical setting, trial was conducted involving 18, 14-15 year old school children, randomly allocated into three interventional groups, Group A (5% Sodium fluoride varnish, Fluoritop-SR varnish), Group B (5% Sodium fluoride - Tricalcium phosphate varnish, Clinpro White varnish) and Group C (5% Sodium fluoride- amorphous calcium phosphate-casein phosphopeptide varnish, MI Varnish) to receive the varnish application. Saliva samples were collected at the end of one hour after varnish application for S Mutans count estimation. Student's paired 't' test and One way -ANOVA tests were used for statistical analyses.

**Results:** Significant reductions in salivary streptococcus mutans count at one hour was achieved with application of B & C varnishes compared to varnish A.

**Conclusion:** amorphous calcium phosphate-casein phosphopeptide and tricalcium phosphate varnish groups exhibited better antimicrobial property against streptococcus mutans compared to Sodium fluoride varnish at one-hour post intervention.

**Keywords:** ACP-CPP, varnish, fluoride, Tricalcium phosphate, Streptococcus mutans

### **1. Introduction**

Dental caries is a widespread, chronic, infectious disease that affects the hard tissues of teeth. It is an external process that starts either at the enamel of the crowns or at the cementum or dentin covering the roots [1, 2]. Fluoride plays an important role in dental caries prevention, primarily due to its effect on the calcified tissues of teeth. However, an important additional preventative effect of fluoride is its ability to reduce acid formation in some bacterial species in dental plaque, including Streptococcus mutans. Fluoride concentrations in plaque can exert inhibitory effect on the oral micro flora [3]. Sustained-release vehicles such as varnishes may exert a long-term prophylactic effect. The agent's efficacy depends on its degree and rate of release from the carrying material. Fluoride and chlorhexidine varnishes have both been found to be effective in this regard [4]. Fluoride varnish application simply involves drying the tooth and painting with a brush applicator. Fluoride varnish can be applied by less skilled dental personnel. Varnish application among high-risk group children shows promising future in prevention of dental caries. According to the latest systematic review done by Cochrane oral health group 2013, young people treated with fluoride varnish experienced on an average of 43% reduction in DMFS [5]. Recently a new remineralization technology based on phosphopeptide from milk protein casein has been developed. The ACP-CPP (amorphous calcium phosphate-casein phosphopeptide) prevents the dissolution of calcium and phosphate ions. It acts as reservoir of bioavailable calcium and phosphate and maintains the solution supersaturated, thus facilitating remineralization.

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Studies have shown ACP-CPP to remineralize enamel subsurface lesions *in vivo* and *in vitro* [6]. Tricalcium phosphate (TCP) has also shown promising results with respect to preventing dental caries and remineralization of incipient carious lesions [7]. Nowadays, amorphous calcium phosphate-casein phosphopeptide and tricalcium phosphate are being incorporated into fluoride varnishes to enhance remineralization potential but very few studies have assessed the antimicrobial effects of such reinforced varnishes against streptococcus mutans. Perhaps addition of amorphous calcium phosphate-casein phosphopeptide and tricalcium phosphate may influence the antimicrobial effects of fluoride varnishes. Studies pertaining to this area are sparse. In this perspective, the present study was conducted to compare the antimicrobial effects of Fluoritop-SR, Clinpro White varnish and MI varnish *in vivo*. The aim of the study was to investigate the antimicrobial property of Fluoritop-SR, Clinpro White varnish and MI varnish and to investigate whether ACP-CPP and TCP interferes with the antimicrobial property of sodium fluoride varnish. The research hypothesis was that there is a difference in the salivary streptococcus mutans counts, after the application of Fluoritop-SR, Clinpro White and MI varnish. The objective of the study was to assess and compare the salivary streptococcus mutans counts at baseline and at one hour after the application of Fluoritop-SR, Clinpro White and MI varnishes *in vivo*.

## 2. Materials and methods

After obtaining ethical clearance from the Institutional Review Board of Bapuji Dental College and Hospital, Davangere, informed consent was obtained from the parents of the study participants after explaining the purpose and procedures involved in the study through a participant information form. Assent was obtained from the children participating in the study. The sample size was calculated using the formula,  $2 \times (Z\alpha + Z\beta)^2 \times (Sp)^2 / (d)^2$ , where, is the minimum expected difference between the groups (based on previously published literature) [8]  $Sp$  is the pooled variance between the groups which was found out to be approximately 8,  $Z\alpha = 1.96$ ,  $Z\beta = 0.84$ , power of study  $(1-\beta) = 80\%$ . Based on the results of the pilot study the minimum expected clinical difference between the three groups was determined. A total of 18 subjects with 6 in each group were selected for the study. Permission to select, examine and collect relevant data from the school children were obtained from the respective school authorities. School children aged between 14-15 years whose parents gave consent to their participation were included in the study. The following subjects were excluded from the study: Children having less than twenty-eight permanent teeth eligible for varnish application, with significant untreated oral diseases like ulcerative gingivitis or stomatitis, extensive tooth decay, acute oral infections, unable to produce adequate amount of saliva for sampling, history of allergy to materials used in the study, undergoing orthodontic treatment, physically handicapped, suffering from systemic diseases and on medications. Children who fulfilled the eligibility criteria underwent baseline salivary streptococcus mutans assessment. Later they were randomly allocated into three interventional groups to receive varnish application.

### 2.1 Randomization

Randomization was done by a separate person not involved in the study. Random numbers were generated using random numbers sequence generator software and the numbers were sealed in the opaque enveloped and coded and handed over to allocator.

### 2.2 The three interventional groups were

- Group A- 5% Sodium fluoride varnish group (Fluoritop SR)
- Group B- 5% Sodium fluoride -Tricalcium phosphate varnish (Clinpro white varnish)
- Group C - 5% Sodium fluoride- amorphous calcium phosphate-casein phosphopeptide varnish (MI varnish)

### 2.3 Varnish application

The teeth were dried completely and isolated with cotton rolls before varnish application. The method of application followed was based on the instructions of the manufacturers of the product. 0.5ml of varnish was applied with a brush applicator on all permanent teeth (excluding third molars) on the teeth surfaces directly and allowed to dry completely for 3-4 minutes. Participants were instructed not to eat or brush for at least 4 hours after varnish application. The subjects were also instructed not to use any oral hygiene aids and also any fluoride dentifrice or rinse or gel for 24 hours after the application of varnish.

### 2.4 Saliva Collection

Five millilitres of unstimulated saliva was collected at baseline and one hour after varnish application from the subjects by asking the subjects to bend down the head and pool the saliva in the floor of the mouth. The pooled saliva was asked to spit into sterile containers. The samples were then sealed, labelled, coded and sent for microbiological analysis. The antimicrobial activity was assessed by disc diffusion method. Colony characteristics formed were studied and the number of colony forming units of *S. mutans* (CFU/ml of saliva) was determined using a colony counter.

### 2.5 Statistical Analysis

The data was compiled systematically in Microsoft excel spread sheet and subjected to statistical analysis using Statistical Package for Social Sciences version 20 (SPSS Pvt Ltd, Chicago, IL, USA). The data showed normal distribution when Shapiro-Wilk's test was applied hence parametric tests were used. Comparison of salivary streptococcus mutans counts within each group at two time intervals (at baseline and one hour after varnish application) was done using student's paired 't' test. Comparison of salivary streptococcus counts between three different groups after varnish application was done using one-way analysis of variance followed by Tukey's HSD Post-hoc test.

## 3. Results

The results of the study indicate that there was a statistically significant difference in the salivary streptococcus mutans counts after the application of Fluoritop-SR, Clinpro White varnish and MI varnishes. At baseline, there was no significant difference between the mean streptococcus mutans counts between the three groups which allowed for valid comparability between the groups post intervention. There was significant increase in the mean streptococcus mutans count at one hour from baseline in the Fluoritop-SR varnish group ( $p=0.026$ ) (Table 1). There was reduction in salivary streptococcus mutans counts after the application of Clinpro White varnish and MI varnish but this was not statistically significant. ( $p=0.345$ ,  $p=0.528$ ) (Table 1). Clinpro white varnish and MI varnish exhibited significantly better antimicrobial effect against streptococcus mutans counts at one hour after varnish application compared to Fluoritop-SR ( $p=0.016$ ,  $p=0.016$ ) (Table 1). However, there was no significant difference in mean streptococcus mutans counts at one hour between Clinpro white and MI varnishes. ( $p=0.376$ ) (Table 1).

**Table 1:** Streptococcus mutans counts after application of three varnishes at baseline and one hour.

Group	Mean baseline salivary streptococcus mutans Counts (x 10 <sup>8</sup> CFU/ml of saliva)	One hour mean salivary streptococcus mutans counts (x 10 <sup>8</sup> CFU/ml of saliva)
Group A	80 <sup>A</sup>	166.67 <sup>A,a,b</sup>
Group B	81.33	50 <sup>a</sup>
Group C	70.17	60 <sup>b</sup>

Superscript lower-case letters indicate significant differences between groups and capital letters indicate significant differences within groups. A- p= 0.026 (students paired' test), a- p= 0.016, b- p = 0.016 (Post hoc Tukeys's HSD test)

#### 4. Discussion

In the present study, there was a significant difference in the mean salivary streptococcus mutans counts between the three groups after the application of Fluoritop-SR, Clinpro White varnish and MI varnishes. The study sample comprised of school boys aged 14-15 year old residing in a common boy's hostel. This was done to minimize the effect of diet and sex variations on salivary composition to certain extent. In order to maximize the preventative action of the varnishes, children aged 14-15 year old were chosen for the present study.

Streptococcus mutans is the most commonly implicated microorganisms in the causation of dental caries and designated as the initiator of dental caries. Since dental caries is an infectious disease, reducing or eliminating the microbial load from the oral cavity is a sensible approach to curb dental caries. Varnish application is usually recommended as biannual professional mode of preventive treatment in the dental office.<sup>5</sup> 0.5ml of varnish was applied with a brush applicator on all permanent teeth on the teeth surfaces directly and allowed to dry completely for 3-4 minutes among the study participants as per the instructions of the manual provided by the manufacturers. Unstimulated saliva was collected from the participants in order to overcome the effect of changing mineral content in the saliva. Mitis Salivarius Bacitracin Agar was used for inoculation of S.mutans as it is considered to be standard media to be used [9]. The antimicrobial effect of fluoride varnishes were assessed by disc diffusion method as this method is considered to be a standard method of assessing the antimicrobial property [9]. A study conducted by Ekenback et al showed, sodium fluoride varnish application containing 22,400 ppm fluoride resulted in significant reduction in streptococcus mutans counts at one hour post application.<sup>8</sup> Contrastingly the results of the present study showed there was an increase in the streptococcus mutans counts after the application of Fluoritop-SR varnish containing 22,400 ppm fluoride. Perhaps the low release of fluoride at one hour may be the reason for increase in the Streptococcus mutans counts. A study done by Reynolds et al showed that fluoride release of 22,400 ppm containing fluoride varnish was less at one hour compared to ACP-CPP varnish and Clinpro white varnish.<sup>6</sup> Few *in-vitro* studies which have tested ACP-CPP pastes for antimicrobial property against streptococcus mutans have shown significant reductions in the counts [10]. This can be attributed to the higher bio-availability of fluoride which leads to decrease in the streptococcus mutans counts. The antimicrobial activity was found to be higher with amorphous calcium phosphate casein phosphopeptide and tricalcium phosphate reinforced sodium fluoride varnishes compared to sodium fluoride, perhaps this may be attributed to increased bioavailability of fluoride at one-hour post application of these varnishes as observed in few studies. Small sample size is the major limitation of the study however the results of the present study can at best be generalized to the commercial pastes used in the study and not to all the sodium fluoride pastes containing ACP-CPP & TCP compounds. Further research is helpful in understanding the antimicrobial activity of reinforced sodium

fluoride varnishes.

#### 5. Conclusion

Amorphous calcium phosphate-casein phosphopeptide and tricalcium phosphate varnish groups exhibited better antimicrobial property against streptococcus mutans at one-hour post application compared to fluoride varnish.

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