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Research Article

EFFECTIVENESS OF SILVER DIAMINE FLUORIDE AND PROPOLIS FLUORIDE VARNISH APPLICATION ON DENTIN WITHIN 60 DAYS

SRI ANGKY SOEKANTO[1,3]*, PRISCILLA DAYANARA[1], HABLI DAVIQ[1], AGOENG TJAHAJANI SARWONO[1], MUHAMAD SAHLAN[2,3]

[1]Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia, [2]Department of Chemical, Faculty of Engineering, Universitas Indonesia, Jakarta, Indonesia, [3]Research Center of Biomedical Engineering, Faculty of Engineering, Universitas Indonesia, Jakarta, Indonesia. Email: sriangky@ui.ac.id

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ABSTRACT

Objective: This study sought to analyze the effectiveness of silver diamine fluoride (SDF) and propolis fluoride (PPF) varnish application on dentin within 60 days.

Methods: We divided 60 blocks (4 mm × 4 mm × 2 mm) of human permanent teeth dentin specimens into six time-based groups: 0 days, 1 day, 7 days, 14 days, 30 days, and 60 days. We then applied 20 µL SDF and PPF varnish to the specimens' occlusal surfaces. For the fluoride test, all specimens were submerged in deionized water, while for the flavonoid test, specimens were submerged in a solution of 20% ethanol. In the treatment group, specimens were shaken for 30 min at 45 rpm (every day) for 0–60 days, depending on group assignment. An ion selective electrode was used to measure fluoride ions and a spectrophotometer with 425 nm wavelength absorbance measured flavonoid absorbance. Dentin microhardness was measured for 30 and 60 days sample using the Vickers microhardness tester.

Results: There was an increase in fluoride ion concentration after SDF and PPF application to the dentin samples, while SDF released more fluoride ions than PPF. Increased flavonoid absorbance was observed after PPF application. Dentine microhardness increased after SDF and PPF application.

Conclusion: SDF and PPF application on dentin is effective within 60 days.

Keywords: Silver diamine fluoride, Propolis fluoride release, Fluoride ion, Flavonoid, Dentine microhardness.

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INTRODUCTION

In 2012, the World Health Organization stated that the worldwide caries incidence rate was 60–90% in children and almost 100% in adults [1]. According to 2013 Basic Health Research (RISKESDAS) results, the prevalence in Indonesia of dental and oral problems is 25.9%, while in 14 provinces, the prevalence of dental and oral problems exceeds the national average. RISKESDAS also showed that Indonesia's Decayed, Missing, and Filled Teeth index is 4.6, which means that the Indonesian population suffers from tooth decay at a rate of 460 teeth per 100 people [2].

Caries are areas of localized destruction of the hard tooth tissue, stimulated by bacterial flora and acid products. Caries occur when there is an ecological imbalance between tooth minerals and oral biofilm because biofilm features microbial activity that decreases mouth pH to a critical limit, causing demineralization to occur [3]. Early mineral loss in teeth can only be seen microscopically, but overtime it can evolve to include visible lesions. Failure to prevent the loss of minerals can lead to cavity formation, potentially causing irreversible bacterial damage to the pulp [4]. Previous research states that caries prevention was most effective when constant low level fluoride was found inside the mouth cavity [5].

One form of fluoride varnish is silver diamine fluoride (SDF), which effectively inhibits active caries in dentin [6]. Another ingredient that can be used to prevent caries is propolis fluoride (PPF), which contains fluoride, which acts as a remineralizing agent, and flavonoids that act as an antibacterial agent. PPF was selected for this study because it contains natural products and is considered safer than SDF. However, detailed research on SDF and PPF has not been widely reported. Therefore, we analyzed the concentrations of fluoride ions emitted by SDF and PPF varnish and measured the absorbance of flavonoids released by PPF varnish. This study also examined the hardness of human dentin after SDF and PPF varnish application relative to the time post-SDF and PPF varnish application (either 30 or 60 days).

METHODS

This was an *in vitro* laboratory experimental research protocol. The samples used in this study consisted of 60 permanent premolars without caries, abfractions, abrasions, or clinical cracks. All premolar teeth were washed with *aquades* and then cut cross-sectionally at 4 mm × 4 mm × 2 mm.

The test materials used were as follows: SDF at 38% concentration and PPF at 10% propolis concentration and 2.139% fluoride concentration. These test materials were made at the Bioprocess Engineering Laboratory, at the University of Indonesia.

The sustained release fluoride and ion-selective electrode tests were performed on days 0, 1, 7, 14, 30, and 60. All samples were shaken for 30 min at 45 rpm, then incubated according to group assignment at 37°C, after which they were subjected to spectrophotometric testing. Hardness was tested using a Vickers microhardness tester. Data on fluoride ion and flavonoid concentrations were analyzed statistically using the Statistical Package for the Social Sciences. Data distributions were tested using the Shapiro–Wilk normality test. If the data distributions were normal, the ratio of each time and each varnish was analyzed using independent *t*-tests to determine between-group differences.

RESULTS

Fluoride ion concentrations in SDF and PPF

Fig. 1 shows the percentage of fluoride ions released following application of SDF and PPF varnish within 60 days. SDF and PPF varnishes showed similar rates of fluoride ion release after 1 day, 7 days, and 60 days.

Fluoride ion concentrations in the SDF varnish treatment and control groups

The teeth in the control group were treated with SDF varnish but were not shaken. We observed a statistically significant difference ($p<0.05$) in fluoride ion concentrations at each time period between the treatment and control groups (Fig. 1).

Fluoride ion concentrations in the PPF varnish treatment and control groups

Overall, the control group showed higher fluoride ion concentrations than the treatment group. Different concentrations were evident at 0 day (Fig. 2). There was a dramatic decrease in fluoride ion concentrations on day 1 post-PPF varnish application, especially in the control group. At 7 days and 14 days post-PPF varnish application, there were almost no differences in fluoride ion concentrations.

Sustained release of flavonoids

All flavonoids were released by PPF varnish shortly after application (Fig. 3). After 1 day, there was a release curve of 41.4%. Thereafter, PPF varnish continued to release flavonoids, with 73.2% as the highest release rate occurring after 60 days. Flavonoid release stabilized after 30 days and the discharge rate differed by only 1.2% between 30 and 60 days (Fig. 4).

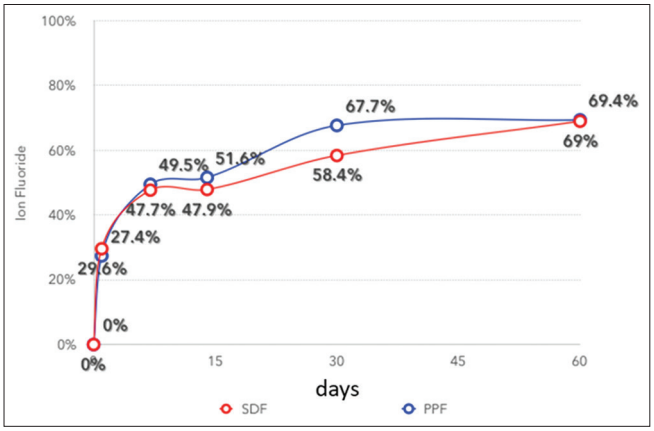

Fig. 1: Percentage of released fluoride ions post-application of silver diamine fluoride and propolis fluoride varnish within 60 days

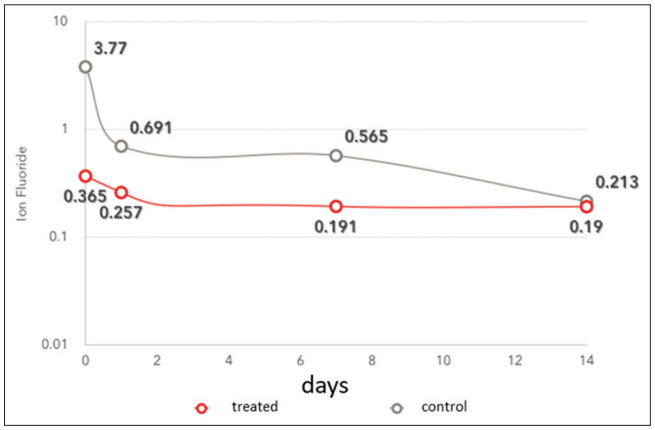

Fig. 2: Fluoride ion concentrations (in ppm) post-application of silver diamine fluoride varnish in the treatment and control groups

The control group exhibited better absorbance than the treatment group; however, all absorbance values decreased overtime. The largest decrease occurred between days 0 and 1. At days 7 and 14, absorbance values were similar (Fig. 5).

Dentin hardness test results

Table 1 shows the results of the independent t-test on dentin hardness post-application of SDF and PPF varnish at 0 day (0.019), 30 days (0.018), and 60 days (0.042). There was a significant difference in dentin hardness ($p<0.05$) post-application of SDF and PPF varnish at 0 day, 30 days, and 60 days.

DISCUSSION

Fluoride ion concentrations associated with SDF were significantly ($p<0.05$) higher than those associated with PPF in each time group. These differences were caused by the fact that the fluoride ion concentration in the SDF varnish was 55,800 ppm while the fluoride ion concentration in PPF varnish was 21,390 ppm, less than half that of SDF [7]. The bonding in SDF was more stable than in PPF.

Fluoride levels influence the incidence of caries overtime. The low fluoride ion levels in saliva, approximately 0.04 ppm, provide a strong protective effect against dental caries [8]. Therefore, low levels of fluoride in saliva are an important parameter when looking at the effectiveness of fluoride agents. SDF and PPF both help prevent caries because even 60 days post-varnish application the fluoride ion concentration was still >0.04 ppm (SDF had a mean fluoride ion

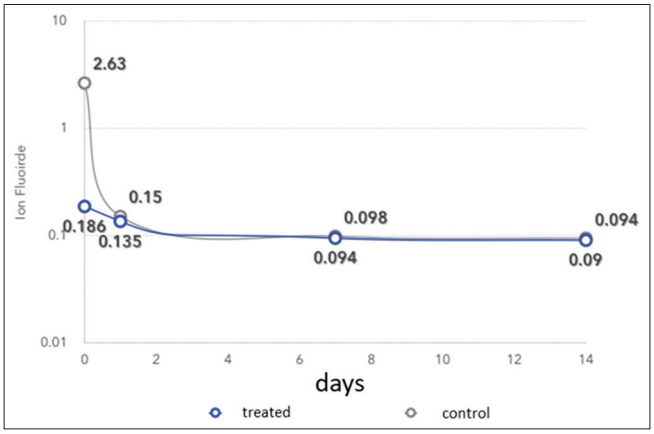

Fig. 3: Fluoride ion concentrations (in ppm) post-application of propolis fluoride varnish in the treatment and control groups

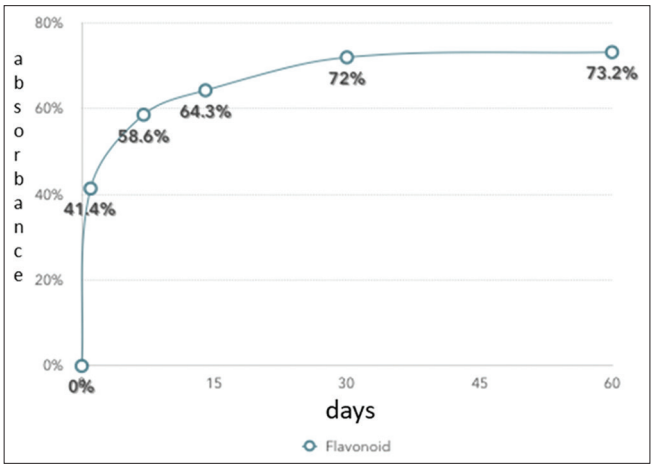

Fig. 4: Percentage of released flavonoids post-application of propolis fluoride varnish within 60 days

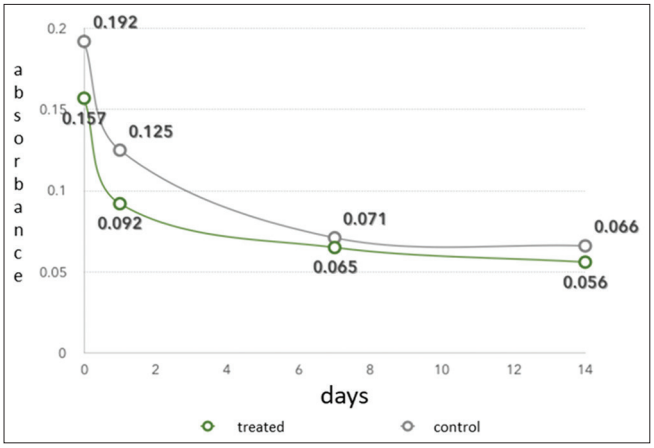

Fig. 5: Absorbance of flavonoids post-application of propolis fluoride varnish in the treatment and control groups

Table 1: Dentin hardness values overtime for both SDF and PPF

Treatment group	Mean (SD)	p Value
0 day		
SDF	64.75±1.23	0.019*
PPF	60.00±2.7	
30 days		
SDF	67.00±2.83	0.018*
PPF	61.00±2.44	
60 days		
SDF	69.00±2.82	0.042*
PPF	73.25±1.71	

*significant difference (p<0.05), SDF: Silver diamine fluoride, PPF: Propolis fluoride

concentration of 0.113 ppm 60 days post-varnish application, while PPF had a mean fluoride ion concentration of 0.057 ppm).

In the SDF group, the fluoride ion concentration was lower than that observed in the control group, which indicates that the fluoride ion concentration in SDF varnish was affected by the shaking activity. The results show that there was a significant difference between each time group (p<0.05). In contrast, the concentration of fluoride ions in PPF varnish in Fig. 3 showed no significant difference between the treatment and control groups in the time groups of 7 and 14 days. We, therefore, deduce that PPF varnish is more stable than SDF varnish. This result contrasts with previous studies, which found that ammonia ions bond with silver ions, forming a complex ion called the silver diamine ion $[Ag(NH_3)_2]^+$, which is very stable [7]. This difference could be caused by the fact that the bond between silver and fluoride is very soluble in water and, therefore, easily broken down [9].

This study describes the relationship between varnish application and tooth remineralization in the oral cavity. This conclusion is supported by previous research, which states that products that increase fluoride ion concentrations will also increase remineralization potential and produce bioavailability of fluoride ion concentration in saliva [10]. The application of fluoride varnish may lead to the formation of a calcium fluoride (CaF_2) layer in enamel, which can be a mineral reservoir and releases fluoride while caries are forming [8].

Flavonoids in the form of quercetin were counted using a spectrophotometer with a wavelength of 425 nm. In this study, we used a solution of 20% ethanol with reference to a previous study that found that flavonoids were not soluble in artificial saliva; thus, flavonoid values cannot be calculated in that medium [11]. Fig. 4 shows that the average absorbance of flavonoids in quercetin form was 0.157 immediately after application of the PPF varnish, which constituted the maximum absorbance rate over 60 days. After this, absorbance continued to decline overtime, stabilizing around 30 days. Flavonoid absorbance at 60 days was 0.042. When viewed in percentage form, the release of flavonoids immediately after varnish application reached 100%. PPF varnish also formed a release curve of 41.4% during the first 24 h and stabilized after 30 days.

The release of flavonoids was higher than with varnish propolis chitosan, which was used in a previous study. This previous study reported that varnish propolis chitosan generated a release curve of 20% over the first 8 h and was stable for up to 24 h. This difference in results could have been caused by the fact that the propolis used in the referenced study was produced by *Tetragonula* sp. bees, which have the highest number of flavonoid compounds [12]. It is known that the chemical content of propolis depends on the region in which it was produced, the presence of resources to harvest the plant resin, the genetic variability of the queen bee, the production techniques used, and the season in which the propolis was produced [13].

Table 1 shows the results of the independent t-test of dentin hardness at 0 day, 30 days, and 60 days after SDF and PPF varnish applications, and our results revealed a significant difference (p<0.05). This result is presumably caused by differences in the fluoride ion concentrations of both varnishes. These results are in accordance with previous studies, which reported that the fluoride ion concentrations in SDF were 52,800 ppm, whereas the fluoride ion concentrations in PPF were 21,390 ppm [14]. Another study reported that there was a 1.85 point difference in the mean value of dentin hardness post-application of PPF varnish and SDF varnish, and a 1.2 point difference post-application of PPF varnish and nanosilver fluoride [15]. Another study applied dentin with NaF at a concentration of 22,800 ppm and showed that the dentine hardness value was 64.78 after 28 days of remineralization [16].

CONCLUSION

Our results show that the application of SDF and PPF on dentine was effective within 60 days. Dentin hardness also increased after application of SDF and PPF varnish.

ACKNOWLEDGMENT

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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