Evaluation of the effect of Silver Diamine Fluoride (SDF) on plaque pH – An In-situ study

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Abstract

Background: Silver Diamine Fluoride (SDF) has garnered much interest because of its ability to reduce and arrest dental caries, excellent antibacterial action, and effectiveness as an efficient alternative to restorative materials, when restoration is not an option. Alteration in dental plaque ecology serves as a major factor in caries development. Plaque pH is a direct reflection of the plaque ecology. But there is no scientific literature available about the effect of SDF on plaque pH.

Aim -To evaluate the effect of SDF on plaque pH.

Methods and Material-In an in-situ study, 20 Study subjects aged 20-30 years wore mandibular Hawley's appliances with an embedded enamel block of 5×5mm. After overnight wear, baseline plaque was collected the following day. The enamel blocks were subjected to application of SDF and a cariogenic challenge, and then plaque samples were collected and pH measured at intervals of at 2, 6, 12 and 24 hours.

Statistical Analysis- Statistical analysis was done using SPSS (Software package for statistical analysis, IBM Corporation, Armonk, New York, US) version 13 software for windows

Results and conclusion: The results showed that even after a cariogenic exposure, the plaque pH did not fall into the acidic range after the application of SDF.

Introduction

Untreated dental caries among children is currently one of the most common issues faced by Paediatric dentists [1.] The untreated dental decay has an impact not only on the oral health of the child, but also on the overall physical and mental wellbeing [2]. So, to prevent such serious consequences it is essential to identify an effective, low-cost treatment alternative that will be available to children with high caries risk and low socio-economic background and limited access to dental treatment [3].

Lately Silver Diamine Fluoride (SDF) has gained much attention due to its ability to successfully arrest dental caries [4]. Clinical studies have revealed that the topical application of SDF at a concentration of 38% is very effective in arresting and reducing dental caries. SDF helps in remineralization of demineralized dentin, and has a possible antiplaque action[5].

Stephen reported that, pH fall after sugar exposure is more in caries-active individuals compared to caries free individuals[6]. Dental caries is associated with increased proportions of certain acidogenic and aciduric bacteria which can demineralize enamel [7]. These organisms grow and metabolize in acidic pH and become more competitive. On the other hand, bacteria associated with enamel health cannot thrive in this acidic environment. There exists a homeostatic mechanism that maintains a beneficial relation between the host and the resident oral microflora. Onset of a plaque mediated disease occurs secondary to the disruption of this homeostatic mechanism [8]. The quality of dental plaque and its caries causing effect largely depends on the pH because, plaque pH is the result of the interaction between the microbiomes in the oral cavity, both of health and disease. If SDF is an effective anti-plaque agent, it should influence plaque pH as well. Hence this study was undertaken with the aim to evaluate the effect of SDF on plaque pH after a cariogenic exposure at various time intervals.

Materials and methods

An In-situ study was conducted in the Department of Pediatric and Preventive Dentistry, Faculty of Dental Sciences, Ramaiah University of Applied Sciences (FDS, RUAS)**.** The sampling method was a convenient sample of 20 Study subjects aged 20-30 years who volunteered to participate in the study and gave consent for the same. Ethical Review

Board approval was obtained and Informed consent from the participants were taken before the start of the study. Inclusion criteria applied was for healthy volunteers aged 20-30 years with a stimulated salivary flow rate > 1ml/min and Un-stimulated salivary flow rate > 0.25ml/min. Individuals who had Fluoride application done within 1 week of the study, allergy to silver, medically compromised individuals and under any medications were excluded.

Selection of volunteers

Volunteers were selected with appropriate salivary flow by estimating both stimulated and unstimulated salivary flow rate. In order to estimate unstimulated salivary flow rate [9], the subjects were instructed to sit in an upright position with the head inclined in a forward position. The saliva that is formed gets collected on the floor of the mouth and could drip into a graduated test tube for 10 minutes and expressed in millilitres per minute. In case of estimation of stimulated salivary flow rate [10], the subjects were asked to chew a flavoured gum for 2 minutes. The saliva is expectorated into a graduated test tube and measured by reducing the weight of the test tube from the total weight of test tube and the saliva formed, and expressed in millilitres per minute. Volunteers with normal flow as per **Table 1** were selected.

Table 1 Reference values for Unstimulated and stimulated salivary flow rate

Flow rate	Unstimulated saliva (ml)	Stimulated saliva(ml)
Normal	More than 0.25	More than 1.0
Low	0.1-0.25	0.7-1.0
Very Low	Less than 0.1	Less than 0.7

Preparation of enamel sections

Freshly extracted permanent teeth were obtained from the Department of Oral and Maxillofacial Surgery, FDS, RUAS. The coronal portion was sectioned along the long axis into buccal and lingual segments using a high-speed diamond tipped disc. The buccal and lingual segments were made into enamel slabs of 5×5 mm size using the diamond disc. The enamel sections were then sterilized with 3% ethylene oxide for 16 hours in the Department of Central Sterile Supply, Ramaiah Memorial Hospital, Bengaluru.

Appliance fabrication

Maxillary and mandibular alginate impressions were made of the 20 volunteers. Casts were poured in dental stone, following which mandibular Hawley's appliances with buccal extensions were fabricated. A window of 6×6 mm was created on the buccal extensions on both right and left side of the appliance using acrylic trimming burs. The enamel blocks were incorporated into this window with Cyanoacrylate adhesive [11].

Measurement of plaque pH

The participants were asked to use non fluoridated tooth paste one week prior to the wearing of Hawley's appliance. Once the Hawley's appliances were delivered to the participants, they were instructed to wear it overnight before the application of SDF. Following an overnight wear of the appliance, the plaque samples were collected from the embedded enamel sections in a 0.5 ml Eppendorf tube and was sent to the lab for pH detection. This constituted the baseline plaque pH before the application of SDF **(T_0)**.

SDF application

SDF applications for all the enamel sections were done outside the oral cavity and the appliance was inserted back in to the subject's oral cavity following the application. The SDF applications were carried out by a single operator. Enamel sections were first dried with a gentle flow of compressed air. One drop of SDF was dispensed on the dappen dish provided by the manufacturer. A micro sponge brush was bent, dipped in to the dappen dish and dabbed on the side to remove excess liquid. It was then applied directly on to the enamel sections on both sides of the appliance. The sections were dried with a gentle flow of compressed air at least for 1 minute, until the liquid became dry. Any excess liquid was removed with gauze or cotton pellet. The appliance was inserted back into the oral cavity and the site was isolated for 3 minutes with cotton rolls [12].

After application of SDF, participants were instructed not to eat or drink anything for 1 hour. After 2 hours, the enamel sections were exposed to a cariogenic challenge. The subjects were given a sugar containing fruit juice (Maaza®) and were asked to swish it around the mouth gently for a period of 5 minutes. Following this plaque sample was collected for pH detection. This constituted plaque sample 2 hours after the application of SDF and a cariogenic exposure **(T_1).** Plaque samples were again collected at intervals of 6

hours **(T_2)**, 12 **(T_3)** hours and 24 **(T_4)** hours. Following the plaque collection, the samples were transported to the lab within 1 hour.

Lab procedure

Preparation: - Dental plaque samples taken in 0.5ml Eppendorf tubes were briefly centrifuged at 1000 rpm for 5 minutes. Samples were suspended in 0.5ml Distilled water and vortexed for 20 seconds at 2500rpm. Plaque suspension was diluted with distilled water in 15ml Falcon tubes.

pH measurement: pH meter (EuTech, India) was calibrated with standard buffer of pH 4.0, 7.0 and 10.0. Electrode was immersed in Plaque suspension and pH readings were recorded.

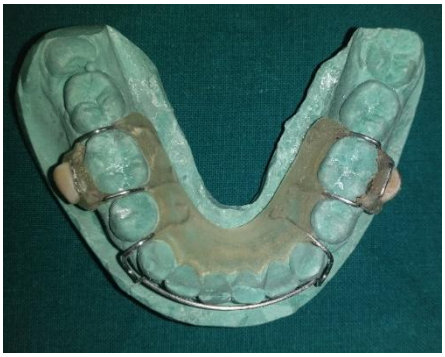

Figure 1: Mandibular Hawley's appliance with embedded enamel sections

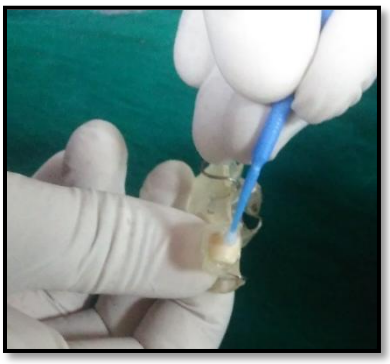

Figure 2: Application of SDF on the enamel section

Statistical Analysis

Statistical analysis was done using SPSS (Software package for statistical analysis, IBM Corporation, Armonk, New York, US) version 13 software for windows.

Results

- The test results demonstrate a difference in the mean pH between different groups. This difference at various time intervals was statistically significant at P<0.001.
- From Table 2, Figure 3, shows that pH of plaque is maintained within the neutral range following the application of SDF even after the exposure to a cariogenic challenge at T_2 (2 hours)

Table 2 Mean plaque pH values at different time intervals and comparison between them using Repeated Measures of ANOVA test

Time	N	Mean	SD	Min	Max	P-Value
Baseline	20	**7.466**	0.125	7.23	7.69	
2 hrs	20	**7.366**	0.101	7.04	7.47	
6 hrs	20	**7.221**	0.162	6.88	7.47	<0.001*
12 hrs	20	**7.097**	0.285	6.54	7.52	
24 hrs	20	**7.041**	0.156	6.68	7.26	

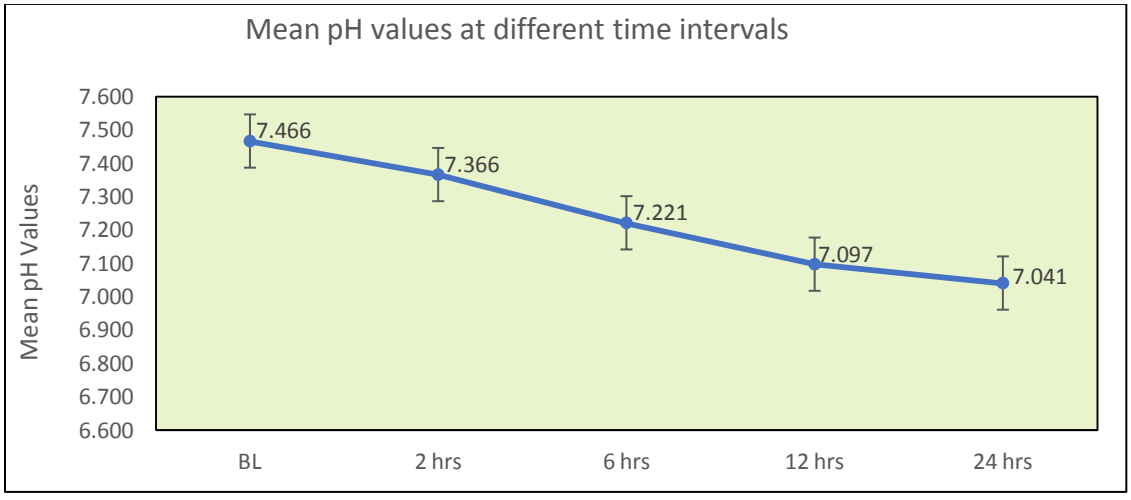

Figure 3 Graph showing mean plaque pH values at various time intervals

The comparison of mean plaque pH between baseline and at time intervals of 2,6,12 and 24 hours shows that:

- The difference between baseline and 2 hours is not statistically significant
- The difference of mean plaque pH between baseline and 6, 12 and 24 hours is statistically significant

Table 3 Multiple comparison of mean difference in pH levels b/w different time intervals using Bonferroni's post hoc Test

(I) Time	(J) Time	Mean Diff. (I-J)	95% CI for the Diff.		P-Value
			Lower	Upper	
Baseline	2 hrs	0.101	-0.039	0.240	0.34
	6 hrs	0.245	0.077	0.413	0.002*
	12 hrs	0.369	0.151	0.587	<0.001*
	24 hrs	0.426	0.270	0.581	<0.001*
2 hrs	6 hrs	0.144	-0.001	0.290	0.06
	12 hrs	0.268	0.031	0.506	0.02*
	24 hrs	0.325	0.183	0.467	<0.001*
6 hrs	12 hrs	0.124	-0.043	0.291	0.29
	24 hrs	0.180	0.052	0.309	0.003*
12 hrs	24 hrs	0.056	-0.135	0.248	1.00

* - Statistically Significant

Multiple comparison of mean difference in the pH between different time intervals revealed that baseline showed significantly highest mean pH as compared to 6 hr time interval [P=0.002], 12 & 24 hrs time intervals, both significant at P<0.001. This was followed by 2 hrs having significantly higher mean pH values as compared to 12 and 24 hrs at P=0.02 and P<0.001 respectively. This was in turn followed by 6 hrs having significantly higher mean pH values as compared to 24 hours at P=0.003. Though, there was a relative

difference in the mean pH between other study time intervals, there was no statistically significant differences observed between them

Discussion

The risk for development of caries lies on the synergistic interaction between the physical, biological, environmental, and behavioural factors related to an individual's lifestyle[13]. These include the presence of cariogenic bacteria in the oral cavity, reduced salivary flow, insufficient exposure to fluoride, poor oral hygiene and cariogenic diet[2].

Stephen in 1940 stated that glucose metabolism by the plaque bacteria result in a considerable drop in plaque pH and plays a major role in caries development[14]. Frequent fall in dental biofilm pH activates acidogenic and aciduric microorganisms that produces acids and in turn would lead to enamel demineralization [15]. Acidogenic and aciduric bacteria like mutans streptococci (S.mutans and S.sobrinus), lactobacilli and some acidogenic non-mutans streptococci can rapidly metabolize dietary carbohydrates into acids, creating a fall in pH which are capable of demineralizing enamel. These organisms are adapted to low pH environment and become more competitive whereas, bacteria associated with enamel health are sensitive to acidic environment[8].

Commensal microbes within the plaque produces organic acids, including acetic, lactic, and propionic acids, as the by-products of fermentation. They dissolve the hydroxyapatite component of enamel and dentine leading to tooth surface breakdown and cavity formation[16].

Dental plaque which are formed in a low cariogenic environment has limited fermentation capabilities and form weaker acids which can effectively buffer plaque pH changes[7]. On the contrary, plaque formed in a highly cariogenic environment produces stronger organic acids that can more readily demineralize dental enamel [8]. So, assessment of acid production from carbohydrate in the dental plaque can be an excellent indicator for assessment of cariogenicity. Studies have shown that dental biofilms, along with its ability to form acids also have ability to form alkali and increase the plaque pH. Reduced ability to form alkaline pH results in increased caries susceptibility[7].

The knowledge about the role that acidogenic and aciduric bacteria play in the demineralization of enamel and further cavitation resulted in the shift of focus from management of decay to prevention of initial demineralization. Then the era of fluorides

started[17]. Fluoride impedes the bacterial production of polysaccharides, and help in preventing tooth demineralization and promoting tooth remineralization[18]. Although the professionally applied topical agents like, fluoride varnishes and gels were beneficial in preventing decay to some extent, none of them proved to be completely satisfactory [17].

Silver Diamine Fluoride introduced in Japan in the 1970's was found to be effective in arresting as well as reducing caries [17]. It was also found to have anti-bacterial action. The success of SDF was demonstrated by more than 20 studies done worldwide. A clinical study conducted by Zhi et al. reported that SDF arrested 91% of carious lesions in 6 months, whereas fluoride arrested 70% lesions in an annual application and glass ionomer arrested 82% of carious lesions [20].

In most of the studies the antibacterial efficacy of SDF is judged by its action against mainly Streptococcus mutans, lactobacilli and Actinomyces, when the activity of other plaque microorganisms are also reflected in the plaque pH[21,22]. Hence, the effect of SDF on plaque pH should be studied. The current study is the first of its kind to assess the same.

Plaque mediated disease develops at a site when there is a breakdown of the beneficial relationship between the resident oral microflora and the host. The cause of breakdown of homeostasis should be recognised and rectified to prevent further episodes of disease process[8]. Hence, it is essential to know if SDF can maintain the internal homeostasis between the host and the resident microflora. From this study, it is quite evident that SDF has an influence on the plaque pH.

Upon a sucrose challenge, as the fermentation process proceeds, the plaque pH drops down drastically within 5 minutes[14]. In the current study when plaque pH was measured at various intervals after application of SDF and a cariogenic challenge, it was seen that the plaque pH still did not drop to acidic level, probably due to the action of SDF supplementing the salivary buffering system.

In the figure 3 we can see that following application of SDF, the drop in pH increases with time, though it was within the neutral range in the study. This indicates that multiple applications of SDF will be more beneficial.

There are certain limitations to this study. As far as caries susceptibility of an individual is concerned, there are many factors that need to be considered such as, Salivary

buffering capacity, diet, oral hygiene maintenance, plaque amount, host defence etc. All these factors were not considered in this study.

To attribute the maintenance of neutral pH within the dental plaque solely to SDF, further in-vivo studies with large amount of plaque samples should be done. The more the pH test solution is concentrated with plaque sample, the more definitive will be the result.

The present study was done on caries inactive individuals. The fermentation of bacteria and acidogenicity of plaque is different in caries active and caries-inactive individuals. So, a study should be done on individuals with increased caries susceptibility and activity to get a more appreciable result. The current study was a first step towards our main goal of assessing, if SDF is capable of modifying plaque acidogenicity. Also, potential for studying the effects of SDF on plaque on a larger sample can provide more reliable results.

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